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CONTENTS

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The Agricultural Chemical Society of Japan.

President : Umetaro SUZUKI.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Further Evidence for the Occurrence of Vitamin E in Soy Bean Oil.

By

Umetaro Suzuki, Waro Nakahara
and Yoshikazu Sahashi.

(Reprinted from Sci. Pap. I. P. C. R. Vol. 24, No. 517, pp 283~286.)

In a previous paper[1] we presented a preliminary report on the occurrence of vitamin E in soy bean oil. The evidence for this conclusion available at that time consisted of the fact that soy bean oil incorporated in a liberal amount to an otherwise vitamin E deficient diet prevented the degenaration of testicles, while other oils, such as peanut oil, cocoanut oil, white sesame oil, palm oil, or blubber oil of finback whale, had no such protective effect on the male germ cells as shown by soy bean oil. Although we were fully aware that the testicular degeneration could not of itself be regarded as a positive proof of vitamin E deficiency, the composition of the diet and the excellent physical conditions of the rats were such that we did not hesitate to conclude that our series of rats fed on oils other than soy bean oil were suffering vitamin E deficiency.

The proof of the existence of vitamin E, however, is incomplete without breeding tests, and it is the purpose of this paper to describe the results of our subsequent experiment which supplies this needed evidence.

Experiment

The synthetic diet used was of the following composition :–

Potato starch (J. P.)	65 g
Fish protein	15 g
Soy bean oil	10 g
McCollum's salt mixture	5 g
Dried yeast	5 g
Biosterol (in olive oil), 1 mg per rat daily, separate from the above mixture.	

Fish proteins and yeast were thoroughly extracted with ether. We did not subject potato starch to ether extraction, since, from the result of our previous experiment, we know that potato starch does not contain enough vitamin E, if any, to interfere with the object of our test.

The soy bean oil used was a guaranteed pure product supplied by the Nisshin Seiyu Co.

The experiment was started with 7 pairs (males and females) of young

albino rats, which were fed on the above diet throughout the entire experimental period of 170 days. In the course of the experiment 3 of the males died of intercurrent infection, and we were forced to allot 2 females to a single male in some cases.

Results

The outcome of the experiment is presented in the accompanying chart (Chart 1) in the form of growth curves with annotations.

Female No. 1 became pregnant toward the 50th day after the beginning of the experiment, but it resulted in abortion. About 30 days later the female

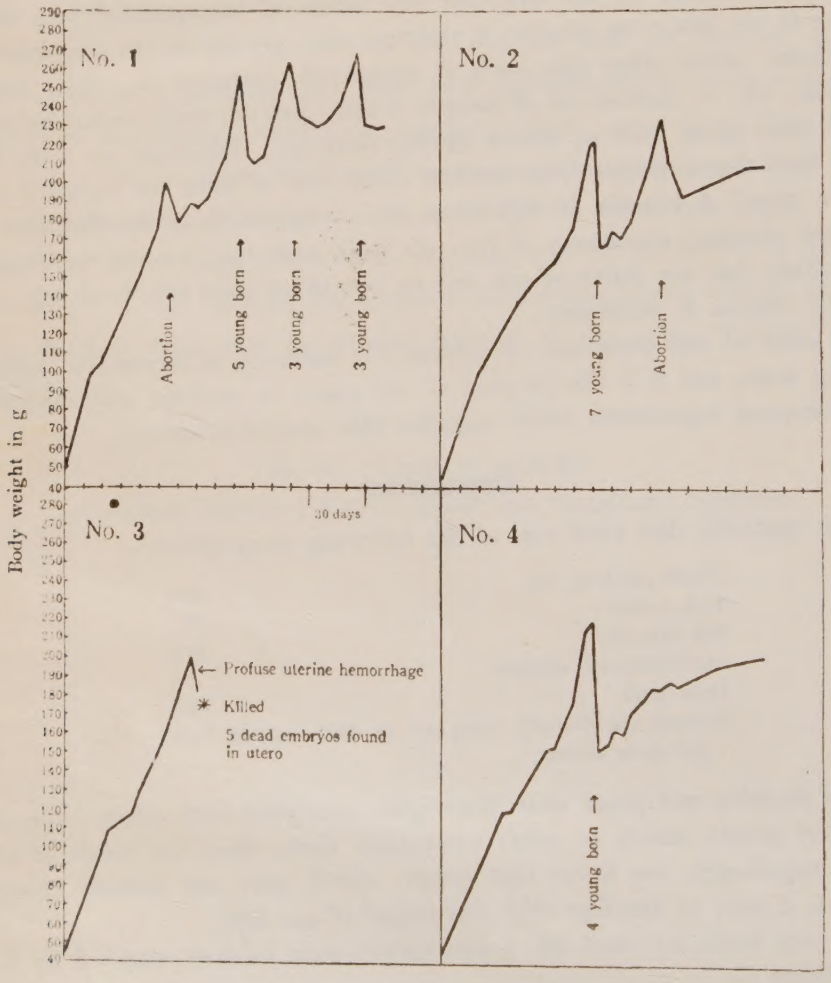


Chart 1.—Growth curves and reproductive incidences of the 4 female rats referred to in the text.

again became pregnant and this time gave birth to 5 young. Some 30 days later it again gave birth to 3 young. The female became pregnant the fourth time and produced 3 young. On all the occasions the young died within two or three days after birth through the failure of lactation on the part of the mother rat.

Female No. 2 gave birth to 7 young 83 days after the beginning of the experiment. The young did not survive due to the failure of lactation. A second pregnancy took place but this resulted in abortion.

Female No. 3 became pregnant about 50 days after the commencement of the experiment. A severe disturbance developed, however, toward the last period of gestation and on the 70th day the animal was found dying from a profuse hemorrhage from the uterus, and was therefore killed. Autopsy revealed 5 fairly large embryos *in utero*, but these were already dead and were undergoing partial disintegration. This is an undoubted case of the interruption of gestation.

Female No. 4 gave birth to 4 young on the 81st day, but the young did not survive. This rat did not become pregnant again for the rest of the experimental period.

Females No. 5, No. 6 and No. 7 did not become pregnant throughout the entire course of experiment. We attach no special significance to these cases since it is quite usual in breeding experiments to find a certain proportion of females that will fail to become pregnant. Growth curves of these females need not be given here.

Four males used for mating were killed at the termination of the experiment, i. e., after 170 days, and autopsies performed. Not only were these rats in excellent physical condition, but they also showed testicles absolutely normal without any sign of degenaration. The testicles weighed 1.1～1.2 g.

Conclusion

The result of the breeding test above described confirmed the conclusion at which we had previously arrived, namely, that soy bean oil contains antisterility vitamin E. We found that female rats were usually able to go through with normal gestation and give birth to litters on a synthetic diet with soy bean oil as the only source of vitamin E. However, to judge from the fact that interruption of gestation occurred on a few occasions, the amount of vitamin E contained in soy bean oil may be relatively small.

Literature

(1) U. Suzuki, W. Nakahara and Y. Sahashi: Sc. Pap. I. P. C. R., **23**, 270, (1934).

Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* Rumph. (VI).

Hydrolytic Oxidation Products of *β–Pachyman* by Conc. Sulphuric Acid and Conc. Nitric Acid.

By

Kenzi Takeda.

(Received August 6, 1934.)

β–Pachyman was treated with a mixture of conc. H_2SO_4, conc. HNO_3 and water at low temperature, which produced no nitro pachyman when the mixing rate of acids and water attained a certain limit as follows, for each 2 g *β*–pachyman :

H_2O	H_2SO_4 (1.840) (g)	Fum. HNO_3 (1.52) (g)	Temp	Time (hr)	Yield of nitrate (g)
6.3	12.9	14.1	17～19°	24	0
7.5	12.4	13.4	〃	〃	0
8.4	11.9	12.9	〃	〃	0
9.5	11.4	12.3	〃	〃	0
8.4	11.9	12.9	10～12°	〃	0.0181

On the contrary, it seems highly probable that oxygluconic acid and glucuronic acid were found to be in these aqueous solutions, as oxydised compounds produced by the complex hydrolytic oxidation, in addition to glucose. They could not have been purely isolated, but two portions separated showed satisfactory positive results enough to identify the presence of the above two compounds.

The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture.

Part. I. The Fermentation Products From Koji Extract.

By

Hideo KATAGIRI and Kakuo KITAHARA.

(Received August 10, 1934.)

The 39 strains of lactic acid bacteria were isolated in the year of 1933 from 38 kinds of moto and in 1934, 18 strains were isolated from 16 kinds of moto and a sample of koji, by plate cultures on chalked maltose yeast water agar.

The 57 strains thus obtained, were classified into two species according to their forms: 37 strains were found to be streptococcus (0.7 μ) and the others were short bacilli ($0.8 \times 1.5 \sim 3.0\ \mu$).

It will be seen in Table I that all the short bacilli produced almost theoretically lactic acid from koji extract, while koji extract was decomposed into not only lactic acid but also alcohol and CO_2 by the streptococci, without any exception, therefore the manner of decomposition of glucose was as follows:

by short bacilli $\cdots\cdots C_6H_{12}O_6 = 2C_3H_6O_3 \cdots\cdots (1)$

by streptococci $\cdots\cdots C_6H_{12}O_6 = C_3H_6O_3 + C_2H_6O + CO_2 \cdots\cdots (2)$

Table I. Fermentation products from koji extract.

Lactic acid bacteria	Average yield of products (%)				Number of strains producing				
	lactic acid	volatile acid	alcohol	CO_2	*l*	*d*	*dl*	*l*+*dl*	*d*+*dl* lactic acid
short bacillus (20 strains)	101.4	2.3	0.7	0	0	7	7	0	6
streptococcus (37 strains)	47.3	2.4	21.7	23.3	36	0	0	1	0
calculated from equation (1)	100.0	0	0	0	—	—	—	—	—
〃 〃 〃 (2)	50.0	0	25.6	24.4	—	—	—	—	—

It is interesting to be pointed out that not only the manner of decomposition of glucose but also the optical rotation of lactic acid produced from koji extract was found to be different between the short bacilli and the streptococci. Table I shows that all the streptococci produced totally *l*-lactic acid excepting only one strain with which the lactic acid consisting of 70% of *l*- and 30% of *dl*-forms was obtained, while *l*-lactic acid was never found

in the products with the short bacilli, since *d*- or *dl*- lactic acid were obtained with each 7 strains and the other 6 strains produced the acid consisting of the above two forms.

The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture.

Part II. Observation of Fermentable Sugars and Some Cultural Conditions.

By

Hideo KATAGIRI, Kakuo KITAHARA and Kanji FUKAMI.

(Received August 10, 1934.)

All the 57 strains of lactic acid bacteria isolated from moto (see Part I) produced acid form pentose, sucrose and glucose, while acid production was hardly detected from glycerol, inulin and mannitol. It will be seen in Table I that arabinose, fructose and maltose were rapidly fermented by almost all the bacteria, galactose was also fermented by most of them although the degree of production of acid was inferior to the formers. Rhamnose, starch, dextrin and lactose were fermented by least number of strains, while fairly large number of bacteria produced acid from *α*-methyl glucoside and salicin.

It will be interesting to note that the fermentability of xylose was remarkably different between the streptococci and the short bacilli, since it was fermented rapidly by all the cocci, while the bacilli were found generally to have no fermenting power of xylose with the exception of three strains by which slight formation of acid was pointed out.

Table I. Kinds of fermentable sugars.

Lactic acid bacteria	Number of strains producing acid from															
	xylose	alabinose	rhamnose	glucose	fructose	galactose	sucrose	maltose	lactose	dextrin	starch	inulin	α-methyl glucoside	salicin	glycerol	mannitol
Streptococcus (37 strains)	37	36	0	37	37	34	37	37	2	1	0	0	25	15	0	0
Short bacillus (20 strains)	3	20	3*	20	19	16	20	18	5	4	4*	0*	4*	5*	0	1

* represents the results of experiments on which 14 strains were under examination.

The maximum acid production was again found to be different between the streptococci with which pH value of fermented koji extract was reached to 3.5 and the short bacilli with which pH=4.0 was observed to be the average value.

The optimum pH value for the multiplication was found to be about 6.0 with all the bacteria, but a remarkable difference in the resistance to alkali was found between streptococci and short bacilli; almost all the former strains grew well on a medium of pH=10.5, while any growth of the latter strains was never observed on such alkaline solution.

It was observed with all the strains that the optimum and limiting temperatures were 30° and about 40° respectively and 35% glucose or 5% alcohol inhibited greatly the multiplication of these bacteria, although their growth was taken place on such media containing 25% glucose or 3% alcohol.

The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture.

Part III. The Fermentation Products from Pentoses and Hexoses.

By

Hideo KATAGIRI, Kakuo KITAHARA, Kanji FUKAMI and Minami SUGASE.

(Received August 10, 1934.)

It is already pointed out (see Part I) that the manner of decomposition of glucose was divided into two classes: all the short bacilli (20 strains) produced lactic acid from glucose according to the equation (1), while glucose was decomposed by all the streptococci (37 strains) into lactic acid, alcohol and CO_2 as was shown in the equation (2).

$$C_6H_{12}O_6=2C_3H_6O_3 \quad (1)$$

$$C_6H_{12}O_6=C_3H_6O_3+C_2H_5OH+CO_2 \quad (2)$$

Any differece was not found between the fermentation products from arabinose by the short bacilli and those of arabinose and xylose by the streptococci, therefore it is concluded all the bacteria decomposed pentoses according to the equation (3).

$$C_5H_{10}O_5=C_3H_6O_3+CH_3COOH \quad (3)$$

The fermentation of galactose or mannose was observed to be taken place in the same manner as was pointed out with glucose. Thus the equation (1) and (2) were found to be applicable not only to glucose but also to all the aldohexoses, for the short bacilli and the streptococci respectively.

The short bacilli were again found to decompose fructose following to the equation (1) however, almost all the streptococci produced mannitol from fructose excepting two strains by which fermentation of fructose was taken place according to the equation (2).

It was suggested that alcohol was directly produced from fructose by the mannitic streptococci by which mannitol was hardly fermented, therefore the new equation (5) which was composed of equations (4) and (2) was proposed for the fermentation of fructose by the streptococci.

$$3C_6H_{12}O_6+H_2O=2C_6H_{14}O_6+C_3H_6O_3+CH_3COOH+CO_2 \cdots\cdots(4)$$
$$28C_6H_{12}O_6+3H_2O=18C_6H_{14}O_6+10C_3H_6O_3+9CH_3COOH+C_2H_5OH+10CO_2 \cdots\cdots(5)$$

The Characteristics of the Lactic Acid Bacteria Isolated from Moto, Yeast Mashes for Saké Manufacture.

Part IV. The Classification of the Lactic Acid Bacteria.

By

Hideo KATAGIRI, Kakuo KITAHARA and Kanji FUKAMI.

(Received August 10, 1934.)

All the 57 strains of the bacteria isolated from moto were found to be true lactic acid bacteria according to their morphological characters, physiological nature of not reducing nitrate etc.

It was pointed out that the bacteria were divided into two species: 20 strains of short bacilli and 37 strains of streptococci, since remarkable differences were found between them not only in their microscopical appearance but also in the manner of fermentation of aldohexoses and fructose, optical rotation of the lactic acid being produced, fermentability of xylose, the maximum acid production, the resistance to alkali and in the reduction of litmus.

When the streptococci were compared with Leuconostoc mesenteroides, Hucker and Pederson, very much the same natures were found between them

as was shown in Table I, therefore Leuconostoc mesenteroides var. Saké was proposed to represent these strains,

It was concluded that the short bacilli were classified as a new species as Lactobacillus Saké, although nearly the same characteristics were found in Streptobacterium plantarum, Orla-Jensen and Lactobacillus arabinosus, Fred, Peterson and Anderson (see Table I) with which remarkable differences were still pointed out in their fermentability of lactose, salicin and mannitol, coagulation of milk, reduction of litmus etc.

Table I. The characteristics of the lactic acid bacteria. (% of the total strains).

Lactic acid bacteria		Streptococcus	Leuconostoc mesenteroides	Short bacillus	Sbm. plantarum	Lactob. arabinosus
Number of strains		37	97	20	9	3
Size (μ)		0.7	0.6-0.9	0.8×1.5-3.0	0.8-1.0× 3.0-8.0	0.5-0.6× 1.2-2.0
Optimum temp. (C)		30	21-30	30	30	30
Acid production from	xylose	100	70	15	20	0
	arabinose	97	87	100	100	100
	rhamnose	0	15	21*	33	67 (slowly)
	glucose	100	96	100	100	100
	fructose	100	98	95	100	100
	galactose	92	97	80	100	100
	sucrose	100	100	100	100	100
	maltose	100	93	90	100	100
	lactose	5	78	25	100	100
	dextrin	3	18	20	89 (slowly)	—
	inulin	0	rarely	0*	33	—
	starch	0	rarely	29*	0	—
	salicin	41	74	36*	100	100
	α-methyl glucoside	68	—	29*	—	0
	mannitol	10	generally	5	100	100
	glycerol	0	rarely	0	44 (slowly)	0
Formation of	CO_2 & alcohol	100	100	0	0	0
	mannitol	95	generally	0	0	0
	slime	100	generally	0	0	0
Milk coagulation		8	20	0	44	100 (softly)
Rotation of lactic acid		l=97 dl+l=3	l —	d=35 dl=35 dl+d=30	dl and rarely d	dl

* represents the results of experiments on which 14 strains were under examination.

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part VII).

On the Pigments of the Green Cocoon (Bombyx mori, var. Seihaku).

By

Masami Oku.

(From the Chemical Laboratory of Gunze Raw Silk Mfg. Co. Ltd., Ayabe-mati, Kyôto-hu, Japan)

(Received August 22, 1934.)

The natural coloring matters of the greenish domestic cocoon (Bombyx mori, var. Seihaku) had hitherto been investigated by several authers, but no one had carried out the complete identification upon the chemical entity of the pigments. I have investigated to isolate the pigments in their purest state and gained new compounds, named by the auther as "Bombycin" (glucoside) and "Bombycetin" (aglucone of bombycin), which were characteristic to have each one atom of nitrogen and show many reactions and absorption bands like flavone derivatives. There were another flavone-like pigments in small quantity which were ether soluble and that found in the mother liquor of bombycin, but the auther could not precisely study them owing to the shortness of material.

Bombycin is a monoglucoside of bombycetin which has a molecular formula near as $C_{29}H_{19}O_7N$ and has three hydroxyl groups. Bombycetin shows two absorption bands of which maximum are near 2700 and 3750 $1/\lambda$ and shows flavone-like reaction. Acetyl bombycetin was obtained through acetylation by acetic anhydride and sodium acetate. It has a molecular formula near as $C_{26}H_{25}O_{10}N$ and has three acetyl groups. Its absorption band shows horizontal portion between 3100 and 3600 $1/\lambda$ and differs utterly from that of flavone or flavonol acetate.

The following items are discussed.

(1) The origin of bombycin can be asked in the mulberry leaves but it must be synthesized biochemically in the body of silk worm (Bombyx mori, var, Seihaku) owing to the fact that the auther fried negative to isolate the nitrogenous flavone-like substances from the leaves. The presence of nitrogenous flavone-like pigments, bombycin and bombycetin, in animal products is, indeed, very noticeable and interesting fact, because no one has yet found flavone-like substances from the animal origin except from the butterfly (Melanargia galatea).

(2) The physiological function of the presence of bombycin in the cocoon layers is perhaps to protect the pupa from the injurious action of the ultraviolet ray.

(3) Bombycin and bombycetin are with bombilupeol deemed as characteristic components found in the green cocoon (Bombyx mori, var. Seihaku).

Experimental.

The yellowish green cocoon layers were extracted by cold 70% alcohol several times and the extract was concentrated and divided into two fractions, ether soluble and water soluble. From the former fraction bombilupeol and ether soluble pigment were searched, to the latter fraction was added neutral lead acetate and the orange yellow precipitates formed were digested with dilute hydrochloric acid. Crude bombycin was precipitated by neutralizing the filtrate by sodium hydroxide. As crude bombycin could not be obtained in any crystal form, it was purified by dissolving in dilute hydrochloric acid and reprecipitated by neutralization. Bombycin is a monoglucoside of bombycetin and melts and decomposes over 300°C and shows many analogous character with flavone-glucosides except that it contains nitrogen. It is brownish yellow powder and shows some imbibitional properties.

Bombycin was hydrolysed by 5% H_2SO_4 and its aglucone, bombycetin, was obtained. From mother liquor of bombycetin, glucose was detected as glucosazone.

As bombycetin could not be obtained in any crystal form by any method, it was first converted into its acetyl derivative and then regenerated by saponification.

Acetyl bombycetin is a slightly yellowish powder which melts between 135～142°C and has a molecular formula near as $C_{20}H_{16}O_4(CH_3CO)_2N$. By inspection of its absorption bands, it has a characteristic feature to have absorption horizontal between 3100～3600 $1/\lambda$ (in alcohol) and differs utterly from that of flavone or flavonol acetates. It is easily soluble in glacial acetic, alcohol and acetic ether; insoluble in water, alkalies and acids. Color reaction by $FeCl_3$ is negative but reduction color by Mg+HCl is after a long time positive (red).

Bombycetin was obtained as follows: after boiling acetyl bombycetin in the mixed solution of glacial acetic acid and few drops of sulphuric acid, the mixture was poured into cold water and the precipitates formed were recrystallized from 50% alcohol. It could not be obtained in crystal form and was yellowish brown amorphous powder. It shows following reaction.

Reduction color by Mg+HCl……intense red.

$FeCl_3$..... .....dark green~dark brown.
Lead acetate .....orange red precipitates.

It is easily soluble in alcohol, aceton and pyridin but insoluble in ether, water and dil. HCl and changes brownish red by touching to alkalies. It melts between 170~180°C and at the same time decomposes by foaming. It has a molecular formula near as $C_{20}H_{19}O_7N$ and has three hydroxyl groups. It could not be methylated easily by dimethyl sulphate and the decomposition substances by alkali fusion could not be obtained in sufficient quantity to identify. It has two absorption maximum 2700 and 3750 $1/\lambda$ (in alcohol) which resemble that of quercetin.

Bombycetin is a flavone-like pigment and yet has nitrogen. That this nitrogen cannot be deemed as mere impurity but as a element of bombycetin is sure from the fact that it is estimated as one atom from both acetyl bombycetin and the regenerated bombycetin.

Ether soluble pigment was obtained from mother liquor of bombilupeol which was hitherto obtained by the auther and published in this bulletin. The pigment has many analogous properties with bombycetin.

Mother liquor of bombycin was concentrated and after adding alcohol, the filtrate was condensed to syrupy and hydrolysed by 5% H_2SO_4. After repeating the extraction with ether, ether solution was evaporated and the residue was dissolved in alcohol. From that solution, brownish yellow crystal appeared in needles in small quantity which showed many analogous properties with bombycetin especially in respect to the absorption bands.

Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* Rumph. (VII).

The Production of Tetracetyl Glucuronic Acid by Acetolysis of *β-Pachyman* in the Presence of Perchloric Acid.

By

Kenzi Takeda.

(Received September 6, 1934.)

In completely acetylated pachyman ($C_2H_4O_2$, about 14%) obtained in the previous study (I) was treated with acetic acid, acetic anhydride and the small amount of perchloric acid, and acetolysis was carried out by controling

the temperature and the time of heating, till the solution became clear, and at which time the contents were taken out and treated in the usual way.

(1) Acetate which was insoluble in hot water but soluble in cold 50% methanol gave a crystalline white powder, having action on Fehling's solution, M. P., 83~86°. Acetic acid, 71.69%. Specific rotatory power, $[\alpha]_D^{20}$ + 9.56° in chloroform and +8.47° in benzene. Molecular weight found was 504 in benzene by the cryoscopic method. This seemed to be a bionic acid or a mixture of acetyl biose and acetyl glucuronic acid.

(2) Acetate soluble in hot water, gave priamatic colourless crystals (Fig. 1) after recrystallization from hot 50% methanol solution, having reduction with hot Fehling's solution and with cold $KMnO_4$ solution. M. p., 103 ~107°. It contained no chlorine. Moreover, the following results were obtained with this crystal.

(a) Saponification products by 1% methanolic ammonia, gave a syrup which did not crystallize. Bial's HCl-orcin, Neuman's orcin and Tollen's naphthoresorcin reactions gave positive results of glucuronic acid.

With 1 g of syrup, phenylosazone test was carried out and no glucosa-

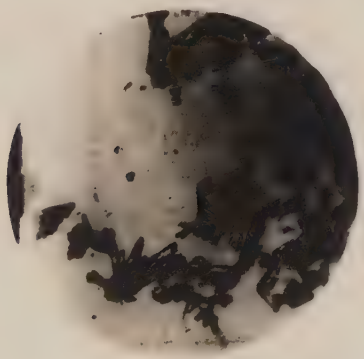


Fig. 1 (1×3)

Fig. 2

Fig. 3 (3×8)

Fig. 4 (3×8)

zone was found. Osazone obtained, however, was a bur like crystal and was soluble in hot water, but with more difficulty than maltosazone. M. p. of osazone, 167～174°. It was soluble in acetic acid, ethanol, acetone and hot water but slightly soluble in benzene and chloroform. Nitrogen content was 14.16% (required, 15.06% for $C_{18}H_{20}O_5N_4$). Molecular weight found was 320 and 345 by Rast's method (required, 372 for $C_{18}H_{20}O_5N_4$).

0.1 g syrup was hydrolysed with 2.5% HCl for 2.5 hours and the resultant syrup gave also the same osazone as above.

Osazones obtained are showed in Fig. 2, 3 and 4.

(b) Acetic acid of acetate found, 83.82% and 83.40% (required 82.87% for $C_6H_6O_7(C_2H_3O)_4$, considering all the acid radicals as acetic acid).

(c) Specific rotatory power, $[\alpha]_D^{20}$ +65.6° in benzene (C, 4.0), +66.2° in chloroform (C, 4.0) and +60.9° in methanol (C, 1.89).

(d) Molecular weight was 423, 392 and 398 in benzene by the cryoscopic method, 370 and 371 by Smith and Young's method (required, 362 for $C_6H_6O_7(C_2H_3O)_4$).

(e) Elementary analysis found was C, 45.90% and 46.02%, H, 5.28% and 5.29% (required, C, 46.40%, H, 5.00% for $C_6H_6O_7(C_2H_3O)_4$).

(f) Acetate was readily soluble in benzene, chloroform, ethylacetate, methanol, acetone and ether, soluble in carbon tetrachloride, acetic acid, toluol and pyridine, almost insoluble in carbon bisulphide and insoluble in water.

From certain of the above figures, one can consider that this may be identical with an acetate which may be accepted as tetracetyl glucuronic acid, though it has never before been reported to have been obtained.

Polysaccharide (VIII).

Ein neues Kohlenhydrat „Xyloglukronid" aus Kadsura japonica Dun.

Von

Kitsuji Nishida, Hideo Hashima und Tokuichi Fukami.

(*Holzchemisches Institut, Kyushu Kaiserliche Universität, Fukuoka, Japan.*)

(Eingegangen am 6, September 1934)

Kadsura japonica ist über Südjapan und Ostindien verbreitet. Der korkrindige Stamm liefert in kaltem Wasser einen gallertartigen Schleimstoff,

aus dem durch Alkohol ein hellbraun gefärbter, voluminöser Niederschlag ausgefällt wird. Der so erhaltene Schleimstoff (2 gr) wurde mit 300~400 ccm Wasser übergossen, darauf wurde er im Autoklav erhitzt und 3 Stunden bei 120~125°C gehalten. Das durchgelaufene Filtrat wurde mit Tierkohle entfärbt. So wurde ein klares, ungefärbtes Filtrat erhalten. Zu diesem wurde nun unter Umrühren ein gleiches Volum $CuSO_4$ Lösung hinzugefügt. Man gewinnt dann eine schön blau gefärbte, voluminöse Fallung, aber sie wird nicht, wie Glukomannan, Xylan, usw. durch Fehlingsche Lösung ausgefällt. Mit alkoholischer Salzsäure zerlegt man diese Aufschlämmung, filtriert das erzeugte Produkt und wäscht es mit Alkohol und Äther. Nach Abgiessen des Äthers und Trocknen im Vakuum auf Phosphorsäureanhydrid ergab sich ein schneeweisses, lockeres Pulver, das mit Jod keine Blaufärbung mehr zeigt und Fehlingsche Lösung nicht mehr reduziert.

Ausbeute und Aschengehalt waren wie folgt:

Holzteil des korkrindigen Stammes	41%
Hellbraun gefärbter Niederschlag, der durch Alkohol ausgefällt wird	0,17%
Schneeweisses Pulver, das durch $CuSO_4$ Lösung ausgefällt wird	0,07%

Wassergehalt (%)	Aschengehalt (%)
8,34	0,10
7,94	0,26
8,00	0,24
8,03	0,22

$[\alpha]_D^{20} = +49{,}0°$ in 5% Wasser.

$[\alpha]_D^{20} = +45{,}8°$ in 5% Wasser nach 24 Stunden.

$[\alpha]_D^{20} = +50{,}6°$ in 5% Wasser mit 2 Tropfen Salzsäure nach 1 Stunde.

Die Makroelementaranalyse ergab:

	Gefunden: %		Berechnet für: $(C_{11}H_{16}O_{10})_n$ %
C	42,26	42,79	42,84
H	6,09	6,10	5,32

Der Xylangehalt wurde nach der Phloroglucinmethode von Tollens und der Kohlensäuregehalt nach dem Lefèvre Verfahren bestimmt und ergab folgende Analysewerte:

	Gefunden: %				Berechnet für $(C_{11}H_{16}O_{10})_n$ %
Kohlensäure	13,16	13,11	12,52	12,72	14,28
Glukuron	52,62	52,43	50,07	50,89	57,12
Xylan	41,45	——	41,53	41,37	42,86

Aus diesen Versuchen geht also hervor, dass das weisse Pulver aus 1-Mol. Xylan und 1-Mol. Glukuron aufgebaut ist. Hier wurden, um zahlenmässige Schlussfolgerungen aus den Angaben herzuleiten, die Falle berücksichtigt, bei denen zu diesem Zwecke Hydrolyse versucht wurde, und wobei

gleichzeitig der Nachweis der Xylose, Glukuronsäure und anderer Kohlenhydrate ausgeführt wurde. Diese Untersuchungen werden zusammen mit denen über Acetylierung, über Methylierung und ausserdem über die Konstitution in einer späteren Mitteilung genauer erklärt werden.

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part VIII).

Isoquercitrin from Mulberry Leaves.

By

Masami Oku.

(From the Chemical Laboratory of Gunze Raw Silk Mfg. Co, Ltd., Ayabe-mati, Kyôto-hu, Japan.)

(Received September 10, 1934.)

The author had already studied the natural pigments of Japanese green cocoon (var. Seihaku) and isolated from it a new pigment, "Bombycin", which showed flavonol-glucoside reaction and contained one atom of nitrogen.

The author tried to isolate bombycin from mulberry leaves but could never find it and, in place of it, isolated fine crystallizable compound, which was identified as isoquercitrin, which is a quercetin monoglucoside (3-glucoside).

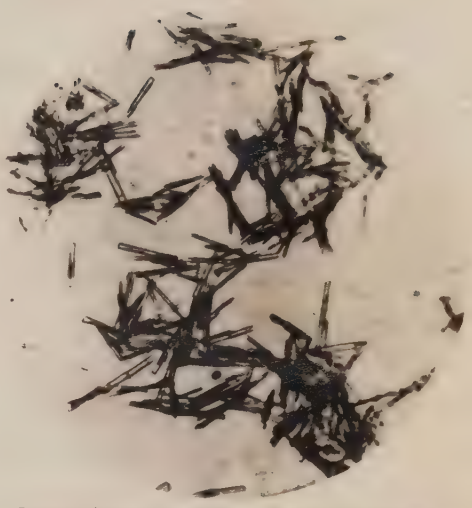
Isoquercitrin was already isolated from brown-husked maize by Sando and Bartlett and from cotton flower by Perkin, but the author was the first to isolate it from mulberry leaves.

Preparation of Isoquercitrin.

The air dried ground powder of mulberry leaves was extracted with boiling 80% alcohol three times and the extract was concentrated, which was shaken with ether and ether soluble substances were rejected. To the water soluble fraction was added neutral lead acetate and the yellow precipitate formed was decomposed by hydrogen sulphide. Seeing that both the lead sulphide and its filtrate contained considerable amount of flavonol glucoside, it was removed by repeated shakings with ethyl acetate. The ethyl acetate extracts were then evaporated and the residue was dissolved in adequate amount of water and then shaken with toluene and left still in a ice box

for several days. The crude glucoside was separated and purified by repeated crystallization from boiling water, finally by dilute aqueous pyridine. Thus about 3.2 g of pure glucoside was obtained starting from 7100 g of air dried leaves (0.05% yield). The glucoside, in its final state of purity, forms pale yellow needle-shaped plates as shown in Fig. 1.

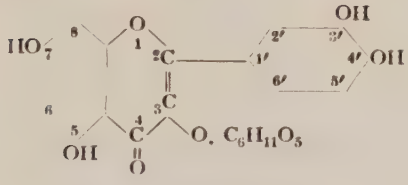
Fig. 1



Isoquercitrin, of mulberry leaves crystallized from dilute aqueous pyridine (×380).

Identification of Isoquercitrin.

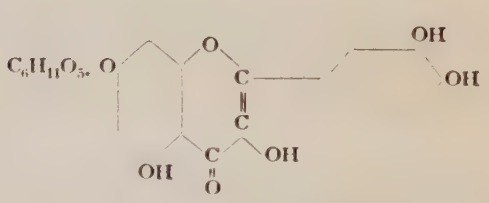
The pure glucoside melts at 220～221° (corr.) and has no water of crystallization and upon hydrolysis by 5% sulphuric acid resolved into quercetin and glucose in molecular proportions. There are two quercetin monoglucosides identified till to-day, cited as follows,



Isoquercitrin

(M. P. 217～219° by Perkin
220～222° by Sando)

Quercetin-3-glucoside



Quercimeritrin

(M. P. 247～249°)

Quercetin-7-glucoside

The author has identified the glucoside as 3-glucoside by methylation and by its absorption spectrum as follows. The pure glucoside was methylated two times by diazomethane ether solution and gained pale yellow needles separated which melted at 150～152°. This methyl glucoside was hydrolysed by 1.5% sulphuric acid and slightly yellow needles separated, which by recrystallization from benzene melted at 196～197°. These results coincided utterly with the data given by Attree and Perkin (J. C. S. 1927, 234), who had proved isoquercitrin to have glucose in 3-position of quercetin.

By inspection of the absorption spectrum of the glucoside and its agglucone quercetin, I have obtained the following results from their absorption curves.

	Absorption maxima (in frequency)	
	glucoside	aglucone
First Band	2850	2700
Second Band	3800	3800

(Concentration : 1/10,000 mol alcohol solution)

These data, too, showed the position of glucose in 3 of quercetin molecule.

Sterilizing Power of Fruits Juice and Condiments on Bac. Typhosus and Vib. Cholerae.

Sogo TETSUMOTO.

(Received September 10, 1934.)

I. Materials.....Fruits juice, Condiments, Strong mineral acid.
II. Experiment. (1) Resisting power of used microorganisms. (2) Experimental method. (3) Detection of test bacteria.
III. Determination of pH of test materials.
IV. Results.
V. Summary.

According to the strong sterilizing action of acids, by Krönig u. Paul's[1], H. Shimokawa's [2] and auther's[3] etc. were reported previously. Fruits, condiments and veverages which are eaten usually raw are almost acid.

I studied on the sterilizing action of fruits juice, condiments and veverages for Bac. typhosus and Vib. cholerae and compared the strength of sterilizing power of many materials for prophylactic purpose.

I. Materials.

I choose such 37 materials as those we like and always use at daily life, avoiding to choose the superior quality.

I. Fruits juice	16 kinds.
II. Seasonings	19 kinds.
III. Strong mineral acids	2 kinds.

Name of material chosen and chief components of sour taste or sterilizing substances are as shown in the following table.

I. Fruits.

	Name of materials	Component of sour taste
Rutaceae	Mandarin orange.....Citrus Aurantium, L. nobiles Makino.	Citric acid
	Chinese citron..... " " , L. subsp. amara, Engl.	"
	Washington Navel orange..... " sinensis. Osbeck.	"
	Lemon..... " medica, L. subsp. Limonum Hook	"
	Yuzu (fresh)..... " Aurantium, L. subsp. Junos. Makino	Citric acid, *l*-malic acid
	Yuzu (shop-worn)..... " " " "	"
Rosaceae	Apple (sour).....Mallus pumilla, Mill. var. domestica	*l*-malic acid, Citric acid
	Apple (not so sour)..... " "	"
	Pear.....Pirus sinensis, Lindl. var. Culta, Makino	*l*-malic acid
	Japanese medlar.....Eriobotrya japonica Lindl.	"
	Peach (Tientsin).....Prunus persica, L. Stokes-var. Tientsin.	*l*-malic acid, Citric acid
	Peach..... " " Stokes.	"
	Sweet cherry.....Prunus avium, L.	"
	Strawberry.....Strawberry variety "Victoria"	*d*-tartaric acid *l*-malic, Citric acid
Vitaceae	Black grape.....Vitis Labrusca vinefera, vit. Camp.	*l*-malic acid, *d*-tartaric acid
	Grape (Koshyu)..... ...Vitis vinefera, L. var. Koshyu.	"

II. Condiments and Strong mineral acids.

	Name of materials	Component of sterilization
Cruciferae	"Wasabi" (fresh and bitter) Eutrema Wasabi japonica	Allylmustard oil
	" (fresh and ordinally bitter) "	C_3H_5CNS
	" (shop worn and not so bitter) "	"
	Raddish (bitter) Raphanus sativus, L.	C_3H_5CNS
	" (ordinally bitter) "	"
Zingiberaceae	Ginger (fresh and bitter) Zingiber officinale Roscoe	Shogaol
	" (ordinally bitter) "	Zingerone
	" (shop worn and not so bitter) "	Zingerone
	"Umeboshi" pickled plum (large)	Citric acid, *l*-malic acid
	" " (small)	NaCl etc,

Sauce	A. imported	{Acetic acid, Citric acid
	B. saled at "M"-departmet	{Cinnamic acid, lemonen
	C. " market	Pepper, ginger etc.
Soy	A. made by "Kikkoman" Co.	{Formic acid, propionic acid, *d*-lactic acid, succinic acid.
	B. " "Yamasa" Co.	Na-caprinate etc.
	C. saled at market	
Vinegar	A. made from saké	Acetic acid.
	B. made by "Riken"	
	C. saled at market	
Strong mineral acids, HCl and H_2SO_4 were the same, so I denote the results of HNO_3 and H_2SO_4	results of HNO_3,	*N*/1000 and *N*/10000 of HNO_3 and H_2SO_4
Control	Ringer's solution	

II. Experiment.

(1) Resisting power of used microorganism.

Degree of resisting power of microorganisms used has great influence on the strength of germicidal activity of materials[1]. I choose next 4 kinds of infectious microorganisms having constant resisting power for phenol aqueous solution referened on G. Reddish's method[4] and standard methods of examining disinfectants. Among Bac. typhosus and Vib. cholerae, I′ and II′ have the very strong resisting power for phenol aqueous solution. On prophylactic purpose I choiced such strong resisting bacteria especially and compared the result of standard test bacteria.

Surviving period (minute)	Phenol aqueous solution			
	I Bac. typhosus (Standard)	I′ Bac. typhosus (Strong)	II Vib. cholerae (Standard)	II′ Vib. cholerae (Strong)
	90 times by weight	90 times by weight	175 times by weight	175 times by weight
5	+	+	+	+
10	+	+	±	+
15	−	+	−	+
30	−	+	−	+
45	−	±	−	−
60	−	−	−	−

+ ····· ···alive −·····perished ±·····sometimes alive and sometimes perished

(2) Experimental method.

We washed fruits and vegetables with sterilized water and dried them at room temperature.

We gathered their juice as cleanly as possible. Juice of "yuzu" was taken by pressing its peel and fruit together.

As the Juice of "umeboshi" (pickled plum) is colloidal state, I dilute it by adding 2 times by volume of sterilized water. Taking 10 c.c. from each material into test tubes and kept them in an incubator at 20°C (within ± 0.5°C). Each of standard bouillon culture of the infectious bacteria was also kept at 20°C. Put 1 c.c. of bouillon culture into 10 c.c. of material and keep them at 20°C. At the certain interval I inoculated microorganisms with the certain platinum wire into standard bouillon of pH 7.0 for Bac. typhosus, and pH 8.0 for Vib. cholerae and I cultivated them 2~3 days at 37°C. Adding 1 c.c. of bouillon culture into 10 c.c. of material, seems too much the bouillon culture comparing to the material, but on prophylactical stand point of view, results of the such case will be more reasonable than the results of small quantity.

(3) Detection of test bacteria from other bacteria.

To detect the bacteria from other, I used many Endo's dish culture for Bac. typhosus and Aronson's dish culture for Vib. cholerae. Colonies of above organisms have been witnessed by morphological investigations and by hanging drop method using 1 : 100 dilution of the agglutination test with immune serum of rabbit of each microorganism. When the experiment comes near the end, and activity of microorganisms becomes faint, living force of test bacteria is suppressed by other bacteria, then the determination of alive or death is very trouble.

Though Endo and Aronson media are special one for Bac. typhosus and Vib. cholerae respectively, but these media prevent the growth of these above microorganisms in some degree.

On this respect I used many neutral agar dish cultures besides of Endo's dish culture for Bac. typhosus, and alkali agar dish culture besides Aronson's dish culture for Vib. cholerae.

III. Determination of pH of test materials.

Each juice of fruits, "wasabi", ginger, or "umeboshi" (pickled plum) differs their pH respectively.

I mixed juice of the same kind of material which appears in the market generally. I determind pH by colorimetric method.

At vinegar, its original state, at Riken vinegar and strawberry juice I diluted 10 times by volume. At soy and sauce 50 times and others are all 5 times I diluted and determined pH of these materials.

Table I. Sterilizing power of various Fruits juice on Bac. typhosus.

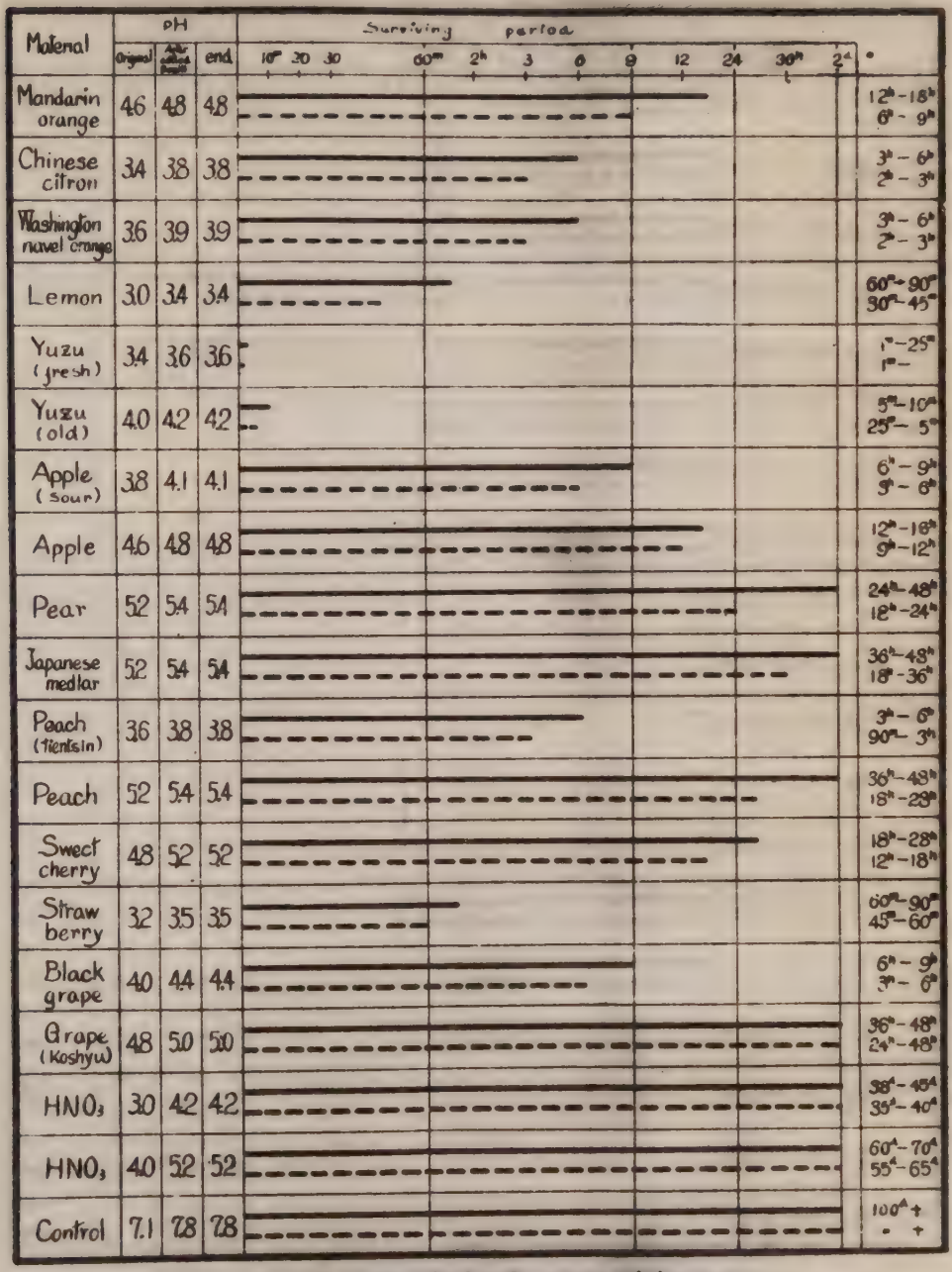
Material	pH: Original	pH: After alcoholic fermentation	pH: end	Surviving Period: 12h 24 36h 2d 5 10 15 20 25 30 35d		
Mandarin orange	4.6	4.8	4.8		8d–10d 7d– 8d	On 7 day fermentation bigins
Chinese citrun	3.4	3.6	3.6		4d– 6d 2d– 4d	
Washington navel orange	3.6	3.8	3.8		6d– 7d 4d– 5d	
Lemon	3.0	3.3	3.3		2d– 3d 36h– 2d	
Yuzu (Fresh)	3.4	3.6	3.6		2h– 3h 90m– 2h	
Yuzu (Old)	4.0	4.2	4.2		6h– 8h 3h– 6h	
Apple (Sour)	3.8	4.0	3.9		4d– 6d 3d– 4d	
Apple	4.6	4.7	4.4		19d–21d 19d–20d	On 19 day fermentation bigins
Pear	5.2	5.3	5.4		30d–33d 30d–31d	On 30 day fermentation bigins
Japanese medlar	5.2	5.4	5.2		30d–32d 30d–31d	On 30 day fermentation bigins
Peach (Rentsin)	3.6	3.7	3.7		5d– 7d 3d– 5d	
Peach	5.2	5.4	5.4		33d–34d 32d–34d	On 33 day fermentation bigins
Sweet cherry	4.8	5.0	5.0		27d–33d 26d–29d	
Straw-berry	3.2	3.3	3.3		2d– 4d 2d– 3d	
Black grape	4.0	4.1	3.8		3d– 4d 2d– 3d	On 2-3 day fermentation bigins
Grape (Koshyu)	4.8	5.0	4.6		3d– 4d 3 – 4d	On 2-3 day fermentation bigins
HNO_3	3.0	4.2	4.2		90d "	After 90 days microg +
HNO_3	4.0	5.2	5.2		100d "	After 100 days microg +
Control	7.1	7.1	7.1		150d "	After 150 days microg +

——— B. typhosus having the strong resisting power.

---------- B. typhosus having the standard resisting power.

h·····hour d·····day +·····survived

Table II. Sterilizing power of various Fruits juice on Vib. cholerae.

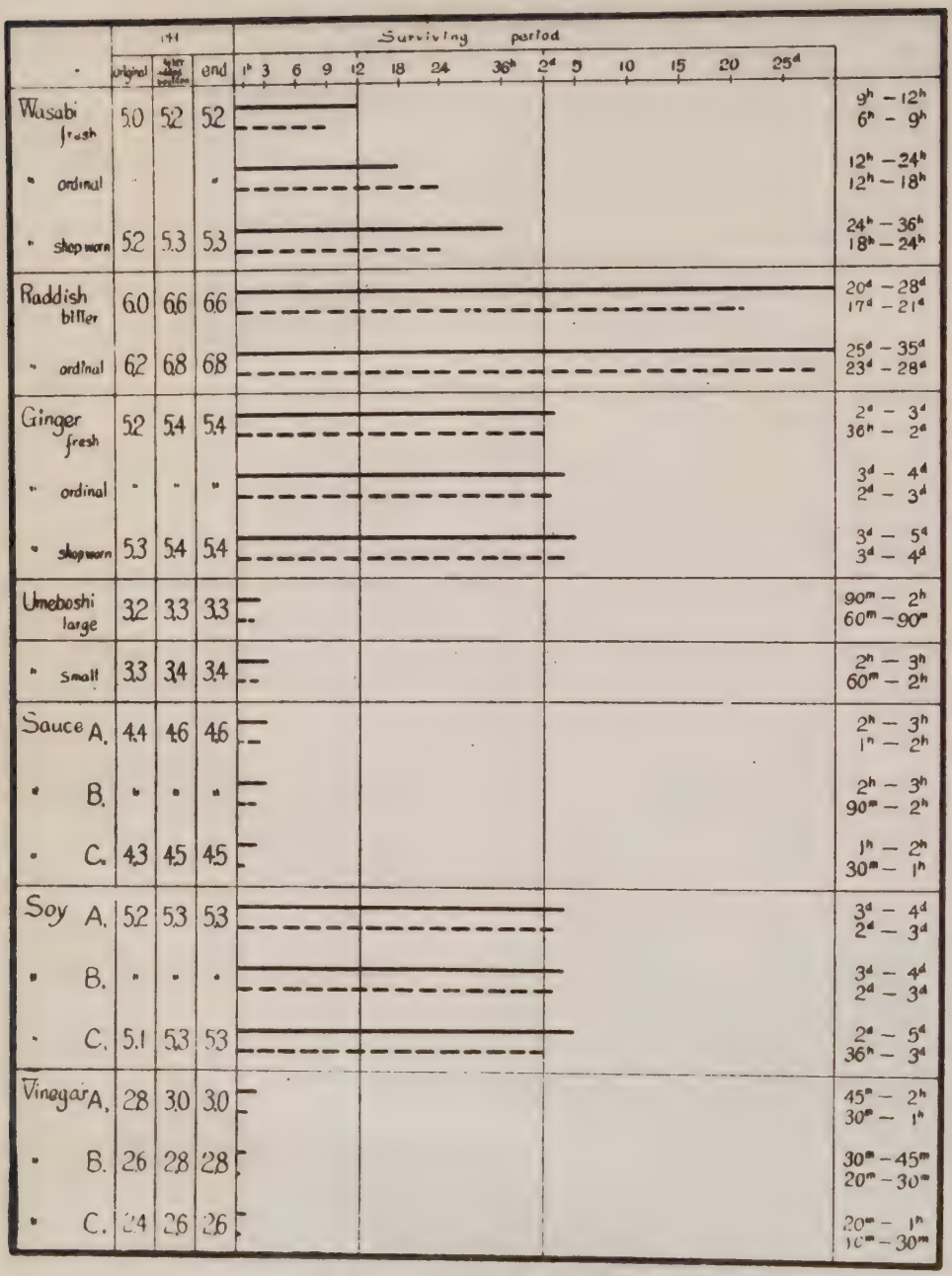


——— V. cholerae having the strong resisting power.

- - - - - - - - - - Vib. cholerae having the standard resisting power.

m……minute h……hour d……day +……survived −……perished

Table III. Sterilizing power of Condiments on Bac. typhosus.

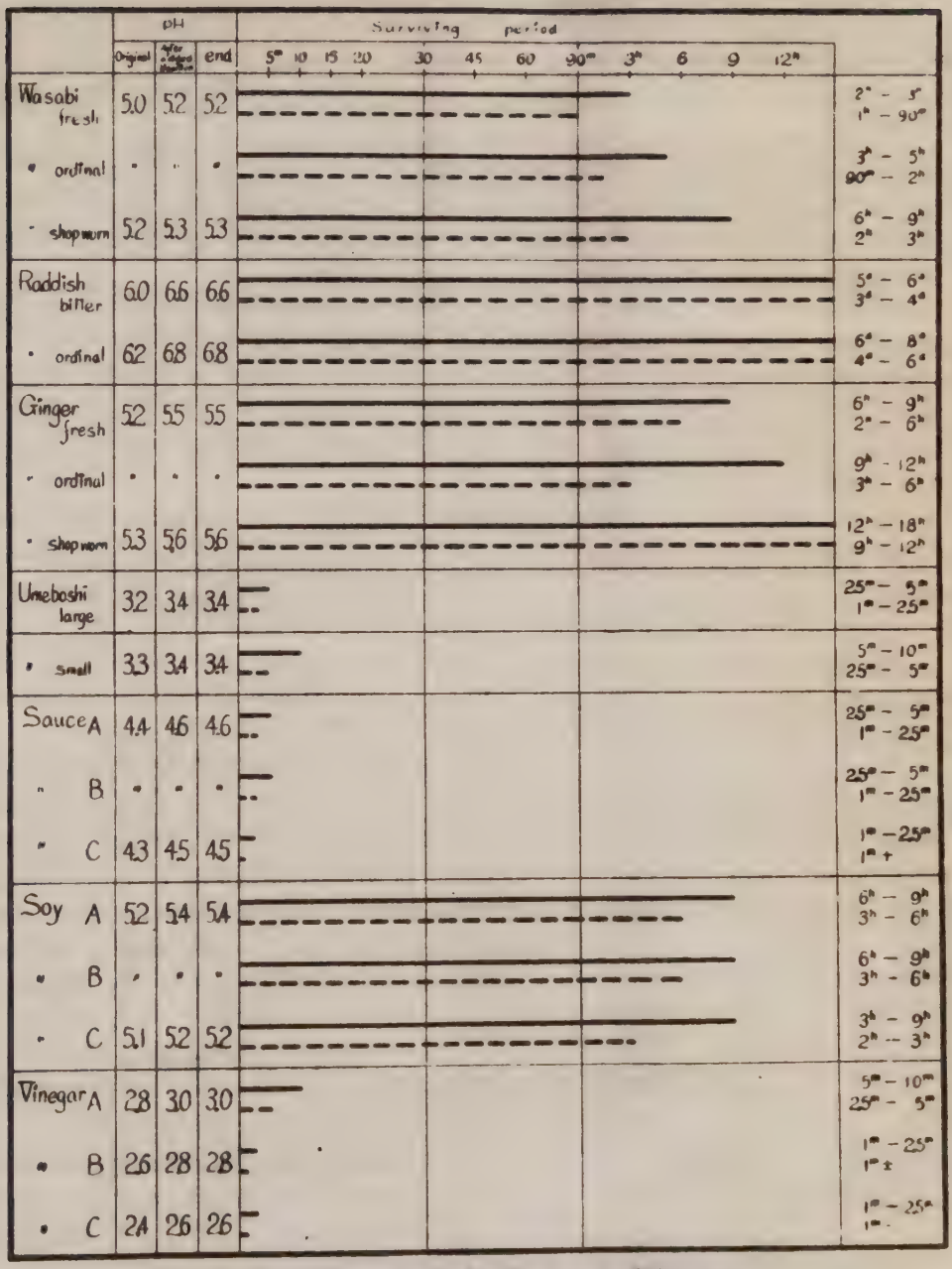


——— B. typhosus having the strong resisting power.

- - - - - - - - - - B. typhosus having the standard resisting power.

h.....hour d.....day +.....survived

Table IV. Sterilizing power of Condiments on Vib. cholerae.



——— V. cholerae having the strong resisting power.

---------- Vib. cholerae having the standard resisting power.

m···minute h···hour d···day +···survived —···perished ±···

IV. Results.

The results obtained are as described in the following table I~IV.

From the above experiments and the results as shown in table I~IV, we know the following facts. In general, sterilizing power of fruits juice for Bac. typhosus and vib. cholerae is strong as proportional to their strength of sourness.

Sterilizing action of these juices due to the action of acid components such as citric acid, *l*-malic acid and *d*-tartaric acid, contained in materials.

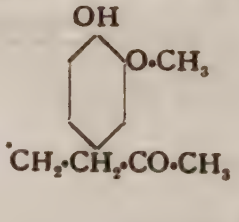
When fruits juice begin fermentation, viability of used bacteria suddenly decays and in relatively short time bacteria perishes.

Change of pH of juice at before and after about fermentation, is relatively small. Accordingly the cause of death of the bacteria is due to the fermentation product of fruits juice rather than the change of pH of the material. Strong sterilizing action of "yuzu" juice is due to the combinated action of citric acid, *l*-malic acid, glucosids and several essential oils such as *d*-limonen, citral and citronellal etc.

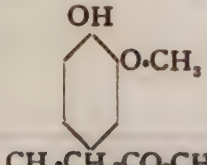
If we compare the sterilizing power of strong mineral acids having nearly the same pH of strong sour or not so sour fruits juice and condiments to that of used materials, we see that test materials have far stronger sterilizing power than strong mineral acids as shown in table I~IV.

By these facts we know that the sterilizing action of fruits juice and condiments is due to the complex action of essential oils and other substances beside pH. By the results noted in table III and IV, we see next facts.

Except raddish juice, many condiments have far stronger sterilizing power than fruit juices generally. The cause of this is due to the complex components other than pH of materials. From the results of "wasabi", ginger and raddish juices, we see that sterilizing power of materials is proportional to the fresh. Among fresh, if we compare the sterilizing power about the degree of bitterness, sterilizing power is proportional to the degree of bitterness. Chief bitter component of "wasabi" and raddish juice is allyl mustard oil C_3H_5CNS and that of ginger are zingerone



and shogaol



$CH_2 \cdot CH_2 \cdot CO \cdot CH : CH \cdot (CH_2)_4 \cdot CH_3$.

Accordingly as chief sterilizing component of "wasabi" and raddish juice, we think allyl mustard oil, and of ginger, may be zingerone and shogaol.

"Umeboshi" (pickled plum) has very strong sterilizing power, its sterilizing power due to citric acid and other unknown substance. The strong sterilizing action of vinegar is chiefly due to the action of acetic acid but besides this, other unknown substance also play strong sterilizing action.

Sauce has very strong sterilizing action. Its sterilizing action due to combined action of acetic acid, citric acid and mustard oil, zingerone and shogaol contained in pepper and ginger.

Sterilizing action of soy is relatively weak. Its sterilizing action is due to the combined action of its component such as formic, propionic, *d*–lactic and succinic acids and Na–caprinate and NaCl. Judging from the result of soy, sauce and vinegar, there is almost no difference about trade mark on sterilizing power.

I express my profound thanks to Dr. Y. Tohyama and Dr. S. Kojima for their kind guidance in my work. And also express hearty thanks to Prof. Dr. K. Shibata, Prof. Dr. Y. Asami and Prof. Dr. H. Nomura for their kind report on the component of several fruits and vegetable juices and etc.

V. Summary.

I studied the sterilizing action of 16 kind of fruit juices and 19 kind of condiments on Bac. typhosus and Vib. cholerae, and the following conclusions are obtained:—

Results are as follows.

(1) "Yuzu" juice has the strongest sterilizing power among such fruits juice as lemonen, mandarin orange and strawberry juices. Juices of Japanese medlar, peach, pear and sweet cherry have weak sterilizing power.

(2) Among condiments, vinegar, umeboshi and sauce have the extraordinally strong sterilizing power.

(3) Condiments have stronger sterilizing power than fruits juice except raddish juice.

(4) On the whole sterilizing power or fruit juices is strong as proportional to their strength of sourness. Their sterilizing action is due to the action of fatty acid such as citric, *l*–malic, *d*–tartaric. Essential oils and a few glucosids have also considerable effect on sterilization.

(5) Sterilizing action of "wasabi" and ginger is very strong. Their sterilizing power is proportional to the degree of bitterness. Accordingly there is close correlation between sterilizing action and bitter component of material.

(6) Vinegar and sauce have very strog sterilizing power. Their chief sterilizing component are complex.

(7) About these complex sterilizing component I will study later.

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Liberation of Vitamin B_2 (Vitamin G) Adsorbed on Acid Earth by the Action of Pancreatin.

By

Waro NAKAHARA, Fumito INUKAI and Shu KATO.

(Reprinted from Sci. paper I. P. C. R. Vol. 24, No. 522, pp 235~334, 1934)

In contrast to the ready adsorbability of vitamin B_2 (=Vitamin G) by a number of adsorbents considerable difficulty was experienced by previous workers in their attempts to release the adsorbed vitamin B_2[1]. As far as we are aware no claim has been made of a successful elution, except by Kuhn, György and Wagner-Jauregg,[2] who used pyridin as eluent. It seems to us, however, that the results of their animal tests are not as convincing as might be desired*.

Recently we found that vitamin B_2 adsorbed by acid earth from liver extract can be liberated through the action of pancreatin. In the present paper we shall first give an account of our experiments on the adsorption of vitamin B_2 by acid earth, and then proceed to describe our method of liberating the adsorbed vitaman B_2.

Adsorption of Vitamin B_2 by Acid Earth.

The liver extract used in this experiment was prepared as follows: 1 kg of fresh beef liver was mashed and thoroughly extracted with 5 litres of 60 % alcohol containing 50 c.c. of 25% sulphuric acid. Extract was separated by filtration and sufficient sulphuric acid added to bring the reaction of the extract to pH 3~4.

To this extract was added white acid earth in the ratio of 20 g of acid earth to 1 litre of extract, and the mixture was maintained at the temperature of 30°C for 2 hours with occasional stirring. The acid earth was then re-

* We hope to take up this question in our later experiment.

covered by filtration and was repeatedly washed with acidified water, with alcohol, and then with ether.

The vitamin B_2 potency of the acid earth treated in this manner was tested in accordance with the usual method. Young albino rats were first maintained on a synthetic basal diet, which was deficient in vitamin B_2, and after a period of two weeks on this diet supplementary feeding of the acid earth adsorbate was commenced and the growth response of the rats noted. The composition of the vitamin B_2 deficient diet was as follows:—

Polished rice powder	75 g
Fish protein	10 g

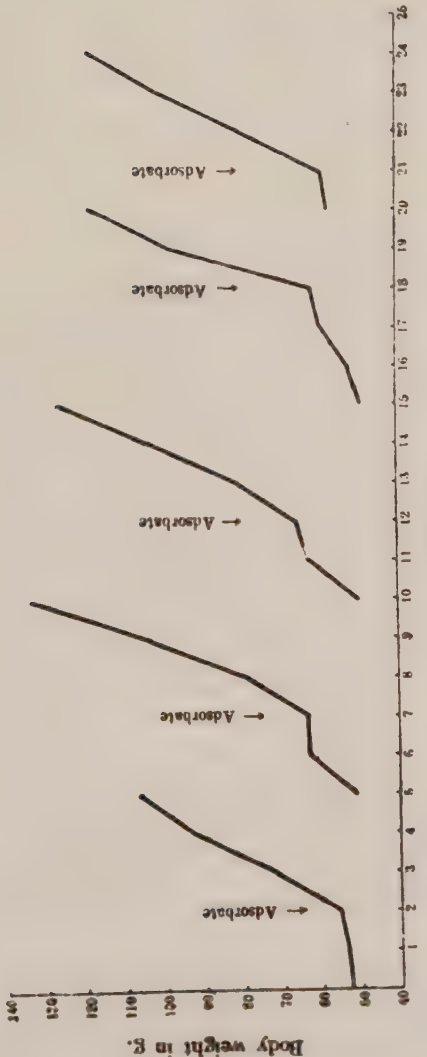

Chart 1—Growth curves of 5 rats showing the growth response to adsorbate on acid earth of liver extract ("activated" acid earth).

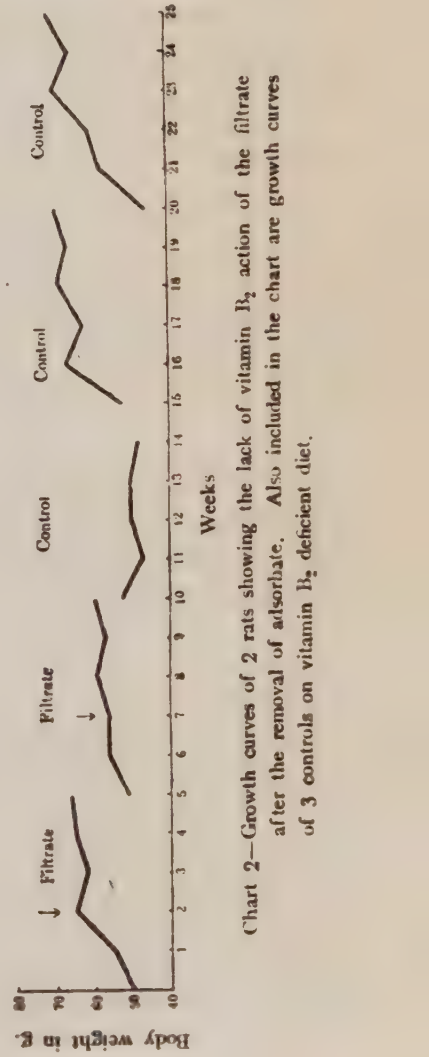

Chart 2—Growth curves of 2 rats showing the lack of vitamin B_2 action of the filtrate after the removal of adsorbate. Also included in the chart are growth curves of 3 controls on vitamin B_2 deficient diet.

Butter	10 g
McCollum's salt mixture	5 g
Oryzanin solution	5 c.c.

Chart 1 represents growth curves of 7 rats on which the effect of adsorbate was tested. In these tests 20 g of the adsorbate ("activated" acid earth) was added to 100 g of basal diet, and all the rats responded very promptly by the typical sharp increase in their body weight.

Chart 2 shows the growth curves of 2 rats on which was tested the effect of the non-adsorbable fraction after the removal of acid earth. This fraction was added to 100 g of basal diet in amounts corresponding to the yield from 1 litre of extract.

The total lack of growth response to the supplementary feeding demonstrates that the vitamin B_2 in the original extract has been entirely removed by adsorption on acid earth.

Growth curves of 3 control rats maintained on the basal diet alone throughout are also included in Chart 2.

Liberation of Adsorbed Vitamin B_2 by Pancreatin Solution.

To 1 per cent solution of commercial pancreatin preparation in 0.1 per cent aqueous solution of sodium-bicarbonate was added the "activated" acid earth in the proportion of 10 per cent, the reaction of the mixture being brought to pH 7.6 by adding sodium bicarbonate. The mixture was kept at 32°C for twenty-four hours under toluol, with frequent stirring, after which the sediment (extracted acid earth) was removed by filtration. The sediment was washed with water, and the wash water was combined with the filtrate, and the whole was concentrated into a suitable volume at a low temperature under reduced pressure in the atmosphere of CO_2 gas. The pancreatin contained in the concentrate was inactivated by heating at 85°C for 20 minutes.

The vitamin B potency of this substance, as well as of the sediment or extracted acid earth, were tested in the same manner as in the preceding experiment.

Chart 3 shows the growth curves of 6 rats on which was tested the effect of different amounts of the substance liberated by the action of pancreatin. The extraordinarily active growth response induced by the supplementary feeding of this eluated substance is remarkable, and it is obvious that the amount recoverable from 5 g of the "activated" acid earth is quite adequate as the source of vitamin B_2.

The extracted acid earth after the removal of eluate was washed repeatedly with distilled water and was tested as to its vitamin B_2 potency in order to determine the extent to which the adsorbed vitamin B_2 was liberated

by the treatment with pancreatin. 20 g of the washed and dried acid earth was added to 100 g of the vitamin B_2 deficient basal diet.

As may be seen from Chart 4, the extracted acid earth still contained some vitamin B_2, showing that the liberation was by no means complete.

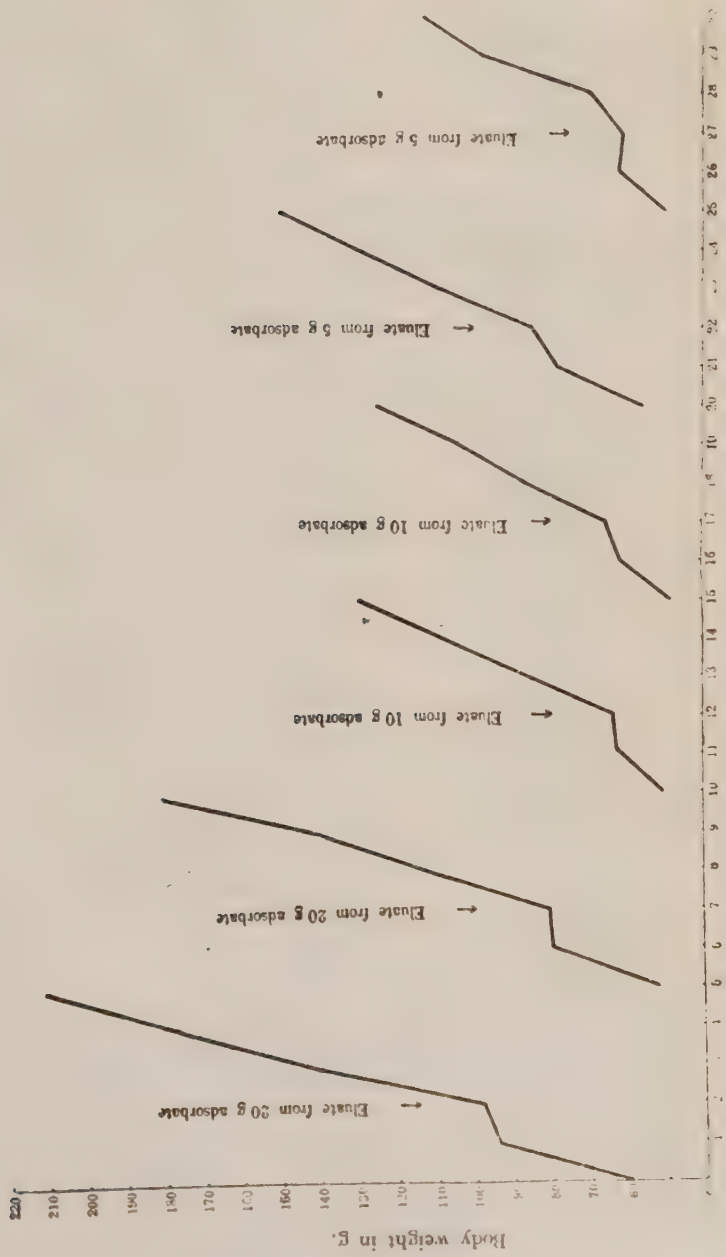

Chart 3—Growth curves of 6 rats showing the vitamin B_2 action of pancreatin eluate in amounts corresponding to yields from 5~20 g of activated acid earth.

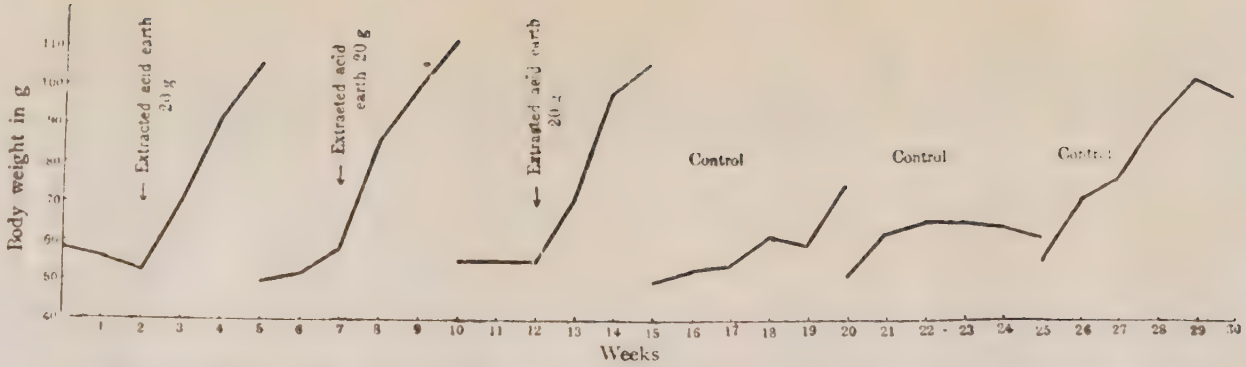

Chart 4—Growth curves of 3 rats on which was tested the effect of extracted acid earth, i.e., the sediment after the removal of the eluate. Growth curves of 3 controls are also included.

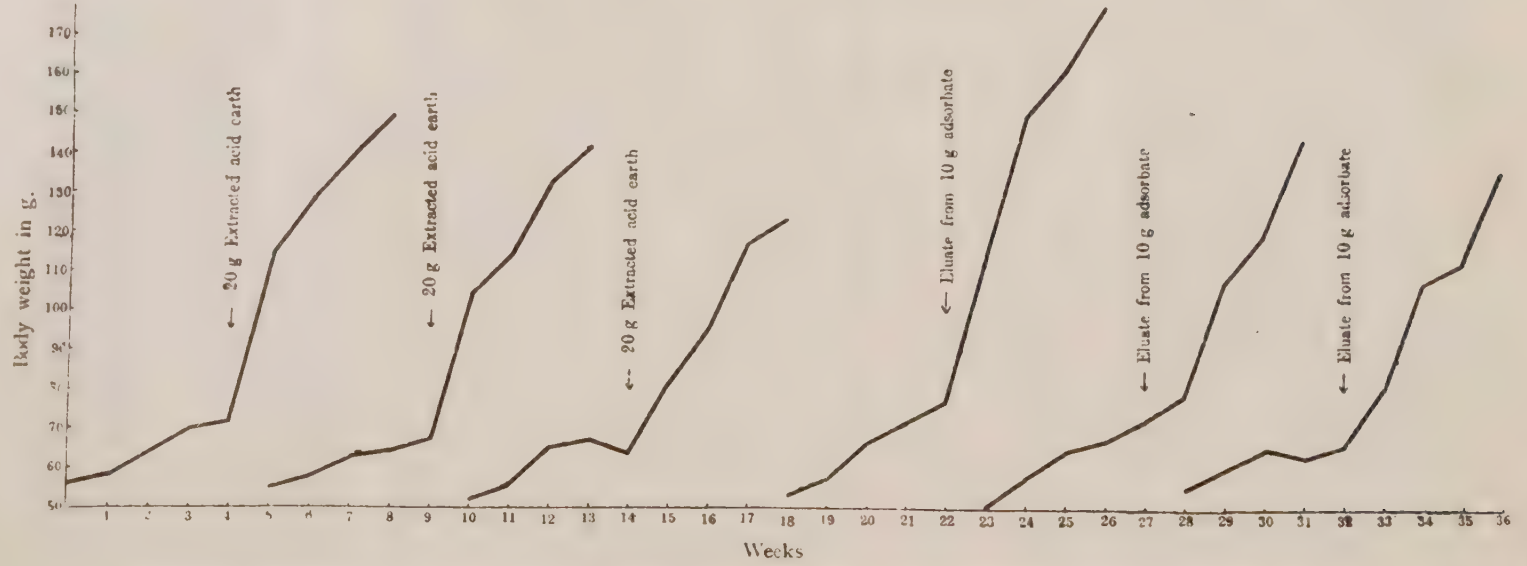

Chart 5—A comparison of the vitamin B_2 action between 20 g of acid earth after the removal of eluate ("extracted acid earth") and eluate from 10 g of activated earth.

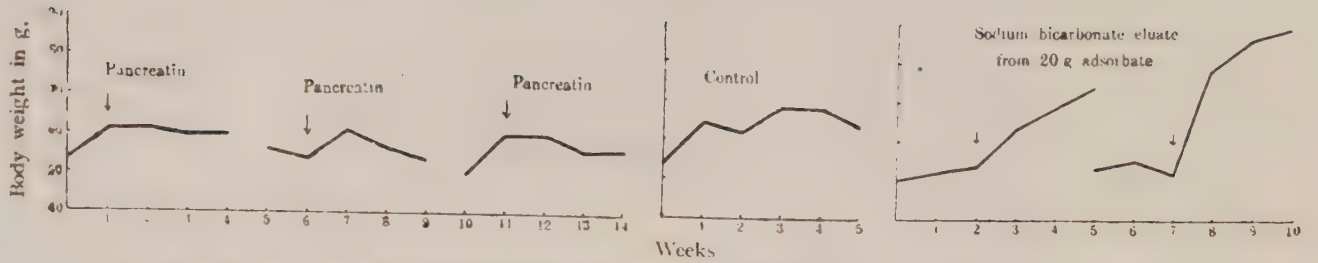

Chart 6—Growth curves of 3 rats showing the absence of vitamin B_2 action in pancreatin itself. In addition a single control on vitamin B_2 deficient diet, and growth curves of 2 rats showing a weak response to sodium bicarbonate eluate are also included.

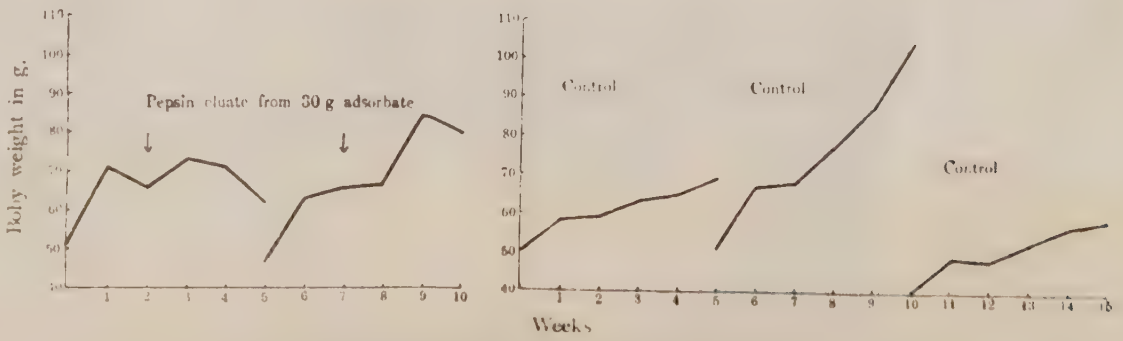

Chart 7—Growth curves of 2 rats showing the lack of growth response to pepsin eluate. Growth curves of 3 vitamin B_2 deficient controls are also included.

The growth response of the rats to a 20 g supplement of extracted acid earth was of about the same magnitude as that which follows a supplement of eluate in amounts corresponding to yields from 10 g or 5 g of "activated" acid earth.

It may be regarded as probable that more than two-thirds the total amount of adsorbed vitamin B_2 was liberated by our method using pancreatin.

An additional experiment showed that this supposition is correct, since, in a parallel test, 20 g of extracted acid earth was found to be slightly less potent than the eluate from 10 g of activated earth (Chart 5).

For control to the above, tests were made also for the possible vitamin B_2 potency of pancreatin itself. 2 g of the same commercial sample of pancreatin as used in the elution experiment was added to 100 g of the basal diet. As shown in Chart 6, pancreatin itself has no growth promoting action.

Needless to say that no satisfactory elution of vitamin B_2 took place when the activated acid earth was treated with 0.1% solution of sodium bicarbonate without pancreatin, although a small amount of the vitamin seemed to dissolve out.into the solution. See Chart 6.

The possibility of the elution of adsorbed vitamin B_2 by pepsin solution was also examined in passing. The procedure of the manipulation was the same as in the case of pancreatin solution, which was simply replaced by one per cent solution of commercial pepsin in 0.15 per cent hydrochloric acid. As may be seen from Chart 7, no satisfactory liberation of vitamin B_2 was attained by the action of pepsin solution.

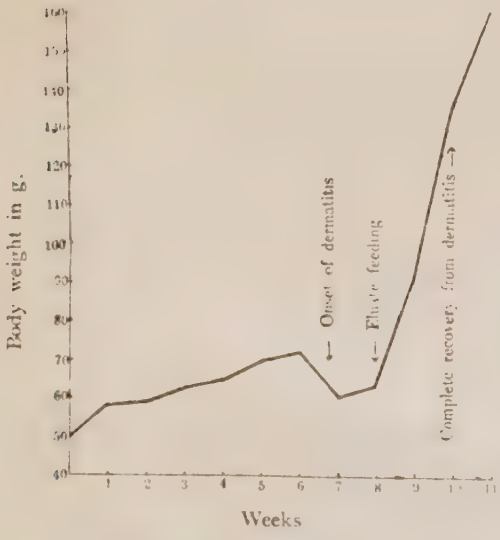

Chart 8—Growth curve of the rat developing pellagra-like symptoms on vitamin B_2 deficient diet, showing the curative effect of our eluated vitamin B_2.

Curative Action of Eluate on the so-called Pellagra-like Condition.

It is well known that vitamin B_2 deficiency produces in certain proportions of the rats pathological conditions somewhat similar to pellagra in man, and that a timely administration of vitamin B_2 brings about the disappearance of these symptoms.

Among our series of vitamin B_2 deficient rats we happened to encounter a single case with definitely recognizable symptoms and we had the opportunity of testing the curative action of our eluated vitamin B_2.

Chart 8 gives the growth curve

of the rat in question, which was fed on the same vitamin B_2 deficient diet as before. It developed marked pellagra-like symptoms during the seventh week and the end of the eighth week we began adding our eluate, in amounts corresponding to the yield from 20 g of activated acid earth, to 100 g of the basal diet. The growth response of the rat was typical.

Figure 1 illustrates the condition of the rat one day before the beginning of the vitamin B_2 supplement, showing dermatitis over the chest and under the arm, and also alopecia around the eye.

Figure 2 represents the same rat 14 days after the beginning of vitamin B_2 supplement, showing a complete recovery from dermatitis.

Figure 1—Rat A, 1 day before the commencement of eluated vitamin B_2 feeding. Note the extreme mal-nutrition and typical dermatitis.

Figure 2—The same rat, after 14 days of eluated vitamin B_2 feeding, showing a complete disappearance of dermatitis.

Comments.

From the physiological point of view, inasmuch as the vitamin B_2 adsorbed on acid earth is utilized by the animal, it is self-evident that the elution of vitamin B_2 must take place somewhere in the alimentary canal. Our present experiments show that this elution must occur in the intestine rather than in the stomach, since the elution takes place *in vitro* under the influeces of pancreatin, but not through the action of pepsin.

Another point of interest in our experimental results is that vitamin B_2

is much better utilized by the animal when supplied in the form of eluate than when given adsorbed on acid earth. This difference in the rate of utilization suggests that no small amount of vitamin B_2 is excreted along with the acid earth which carries it. Our method of liberating vitamin B_2 from the adsorbent therefore offers the advantage, not only of doing away with the ingestion of bulky adsorbent, but also of facilitating the utilization by the animals of the vitamin B_2 administered to them.

Conclution.

Vitamin B_2 in liver extract is completely absorbable by acid earth, but from the experience of previous workers, it may be expected to be difficult to eluate it by ordinary means.

In the foregoing experiments we found that the adsorbed vitamin B_2 can be satisfactorily released by the action of pancreatin. Moreover, it was found that the vitamin B_2 thus released is more readily utilized by the animal organism than the same vitamin adsorbed on acid earth.

Our cordial thanks are due to Professer U. Suzuki, M. I. A., for his kind advice and helpful criticisms given us in the course of this work.

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Isolation of Vitamin C from Vegetables and the Relations between Vitamin C and Ascorbic Acid*.

By

Suttekiti Maruyama.

(Received September 19, 1934.)

In recent years no chapter but that of C in the vitamin research has made so wonderful a development.

Szent-György[1] first isolated in 1928 from suprarenal cortex a reducing crystalline substance consisting of an elementary composition of $C_6H_8O_6$, but,

as it seemd to be an isomer of glycuronic acid, he called it "Hexuronic Acid". This compound was obtained by him also from orange and cabbage.

Tillmans and his collaborators[2], noticing the properties of hexuronic acid reported by Szent-Györgyi, made a supposition in 1932 that Szent-Györgyi's hexuronic acid might be identical with vitamin C. This, in fact, was based upon their own theory that both the reducing capacity and vitamin-C activity in vegetables were always parallel.

Almost simultaneously and independently, Szent-Györgyi[3] made a remarkable observation that his hexuronic acid could prevent or cure experimental scurvy in guinea pigs by giveng them *per os* a daily dose of 1 mg of the acid. He then claimed that hexuronic acid would most probably be identical with vitamin C.

This finding by Szent-Györgyi was traced and confirmed by many other workers who showed that hexuronic acid obtained not only from suprarenal cortex, but also from such different vegetable sources, as lemon juice[4], hawthorn[5], and a Hungarian red pepper[6], had possessed nearly the same antiscorbutic activity.

This active compound is now called "Ascorbic Acid" by Szent-Györgyi.

With regard to the question whether or not ascorbic acid and vitamin C are identical, there are valuable works as follows: Demole[7] stated that four different compounds though mutually related, namely ascorbic acid, its sodium salt, its reversible oxidation product (dehydro-ascorbic acid) and ascorbic acid regenerated from the latter, (the first two had been recrystallized several times) had nearly the same antiscorbutic activity in a daily allowance of 1 mg. Micheel and Moll[8] made an excellent demonstration showing that monoacetone derivative of ascorbic acid, when administrated subcutaneously, was completely inactive, in spite of the fact that ascorbic acid regenerated from it by saponification, indicated the same activity as the initial ascorbic acid, in oral as well as in subcutaneous administration. Svirbely and Szent-Györgyi[6] pointed out that the said acetone derivative of ascorbic acid, in oral administration, was from 1/3 to 1/2 as active as ascorbic acid itself. More recently, T. Reichstein[9] announced that his synthesized ascorbic acid was as active as natural ascorbic acid. This evidence may be a decisive one in favour of the identity of vitamin C with ascorbic acid. However, the data concerning animal experiments are not as yet reported.

This was the situation in the research of vitamin C at the beginning of the present work.

In Japan, however, ascorbic acid, so far as the author knows, has not been isolated in crystalline form, to say nothing of testing its anti-scorbutic effect. This led him to undertake experiment along with this line of research.

First, an attempt was made to obtain ascorbic acid from suprarenal cortex as starting material, by following as strictly as possible the procedure recommended by Szent-Györgyi [1], but in vain.

This failure on the part of the author may have been due to his unskillfullness. The procedure, as then modified by him, finally gave satisfactory results; a reducing crystalline substance so obtained, perfectly coincided in chemical and physical properties with ascorbic acid reported by Szent-Györgyi or by Tillmans.

This first experience led him to study further as to whether ascorbic acid could be found in different kinds of native vegatables already known to contain vitamin C, such as "Daikon" juice[10] (Daikon, *Rhapanus Sativus* L.), "Natsumikan" juice[10] (a kind of Japanese lemon, *Citrus Aurantium*, L.) and Japanese green tea.[11] In fact, one and the same ascorbic acid could be obtained in crystalline form from all these materials by means of his own isolation method.

The first two kinds af ascorbic acid preparations, namely, preparation from suprarenal cortex of oxen and that from Daikon juice, were examined for their antiscorbutic activity on guinea pigs. The result showed that both preparations were equally effective by feeding them with a daily dose of 0.8 mg of the preparation.

After all these findings, the author has come to think that ascorbic acid taken from the other materials is also effective.

An animal experiment is now in progress to verify this possibility. So far as the results hitherto obtained are concerned, ascorbic acid preparation from Japanese green tea is found to be as effective as those from the other two materials. 4 guinea pigs that have been fed on both vitamin-C-deficient basal diet and ascorbic acid obtained from Japanese green tea as supplement to the basal diet, have survived for 5 weeks from the beginning of administrating the acid, while four negative control animals fed on the basal diet alone, died of severe scurvy within the first several days. Those surviving animals are now gradually increasing their weight and are recovering from scurvy.

As soon as the animal experiment employing ascorbic acid taken from Japanese green tea, reveals a satisfactory development, the author expects to report the results later on, more in detail.

Experimental Part.

*Isolation of Ascorbic Acid from Suprarenal Cortex of Oxen.**

The author has succeeded in obtaining ascorbic acid in a satisfactory

* It is needless to say that in isolating ascorbic acid from natural stuffs, the author in any case carried out all of the necessary operations in an atmosphere of carbon dioxide as much as possible and at a temperature as low as possible. In the course of this and other isolation experiments described in the latter part of this paper, ascorbic acid existing was quantitatively determined from time to time by Tillmans' titration method with dichlorphenol-indophenol, in order to justify any operations tried. (refer to: J. Tillmans: loc. cit.)

manner as given below:—

2,100 c.c. of methyl alcohol mixed with 0.21 c.c. of 10 per cent aqueous solution of sodium cyanide, was bubbled, while being cooled in ice water, from carbon dioxide for half an hour. To this cold mixture was then added 700 g of fresh suprarenal cortex freshly cut into small pieces with a meat chopper, and a hot saturated aqueous solution of 11 g of barium acetate. The whole mixture was then cooled and bubbled for another half an hour. The mixture was then pressed out through cotton cloth.

The turbid filtrate was clarified through filter paper, and to the filtrate was added a hot saturated aqueous solution of 110 g of lead acetate all at once, and then cooling and bubbling were continued for an hour. The lead salt which separated was collected on a suction funnel and was carefully pressed and washed with two small portions of cold methyl alcohol to which were added a few drops of the above-mentioned lead acetate solution. The lead salt was ground down, placed into a little amount of cold water and promptly decomposed by adding 20 per cent sulphuric acid in a slight excess

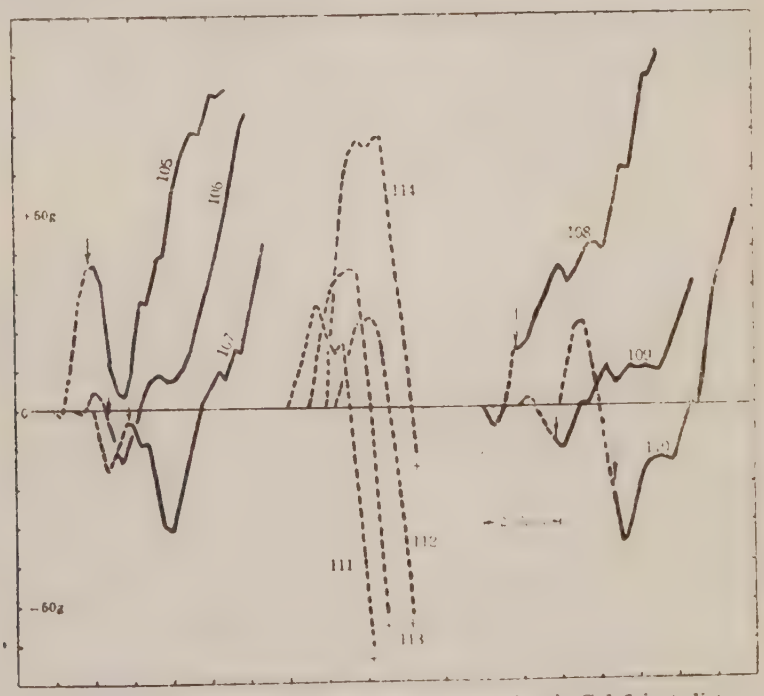

Chart 1—Growth-curves of guinea pigs fed on vitamin-C deficient diet with and without ascorbic acid supplement. Animals No. 105—107 took daily dose of 0.8 mg of the ascorbic acid preparation from suprarenal cortex; No. 108—110, daily dose of 0.8 mg of the preparation from Daikon juice.
----------: basal diet alone, ————: basal diet and supplement.

until a test sample of the mixture distinctly became acid against thymol blue paper. Then the precipitate of lead salt was separated with a centrifuge and was washed twice with small portions of cold water.

The combined clear solutions were then evaporated in vacuum at a temperature not higher than 30°, to be dry. The dry residue was extracted by methyl alcohol, which dissolved nearly all of the residue but the inorganic salts. These salts were separated by filtration and washed with methyl alcohol.

The methyl alcoholic filtrate was again concentrated under rejuice and melting at 178—180°.

10 guinea pigs were fed on the basal diet deficient of vitamin C. The first symptoms experimental scurvy were observed within about 10 days:— abnormal liveliness or passive movement, sensibility to pressure, interruption of growth or decrease of weight, etc.

On the 9th day they were divided into 3 groups, of which the first included 3 animals, No. 105, 106 and 107, the second 3 animals, No. 108, 109 and 110, and the 3rd 4 animals, No. 111, 112, 113 and 114. In the 1st group each one was administrated every day *per os* with 0.8 mg of ascorbic acid preparation from suprarenal cortex, in the 2nd with 0.8 mg of that from Daikon juice and in the 3rd, as negative control, with no such preparation as supplement to the basal diet. The beginning of the administration of the test substances in the chart is indicated with a small arrow. All animals in the 1st group and in the 2nd group soon restored their usual health and normal growth. At the end of experiment, which, owing to the lack of test material, continued only for 45 days, they showed their complete healthiness. They were then chloroformed and subjected to autopsy. The section, in fact, revealed no symptom of scurvy, only indicating a trace of swell on the joints between bones and cartilages of ribs. So, if the process of feeding had been kept on, this trace would have also been cleared off.

The 4 animals in the 3rd group, on the contrary, gradually decreased their weight, and soon died. In the autopsy severe symptoms of experimental scurvy were observed as: swollen condition in the boundaries of bones and cartilages of ribs, swollen condition in region of knee-joint, subcutaneous and intramusculary hemorrhages, etc.

From the foregoing result of therapeutic experiments there is no doubt that so far as ascorbic acid from suprarenal cortex of oxen and ascorbic acid from Daikon juice are concerned, they were anti-scorbutically effective on experimental scurvy in guinea pigs in a daily dose of 0.8 mg.

Ascorbic acid preparations thus tested may be as yet somewhat impure, when it is considered that their melting point is lower than that reported on

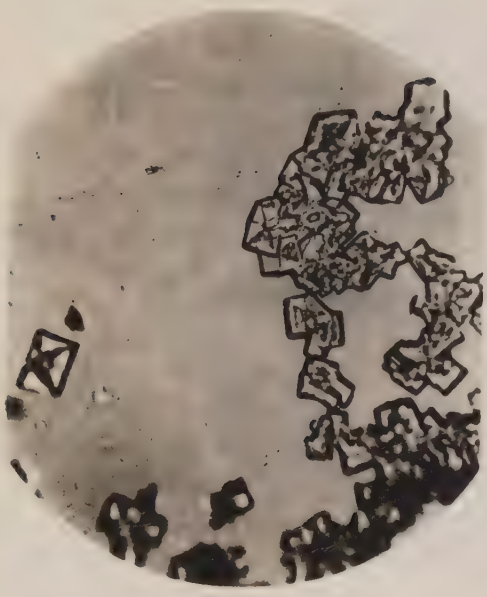

Photo. 1

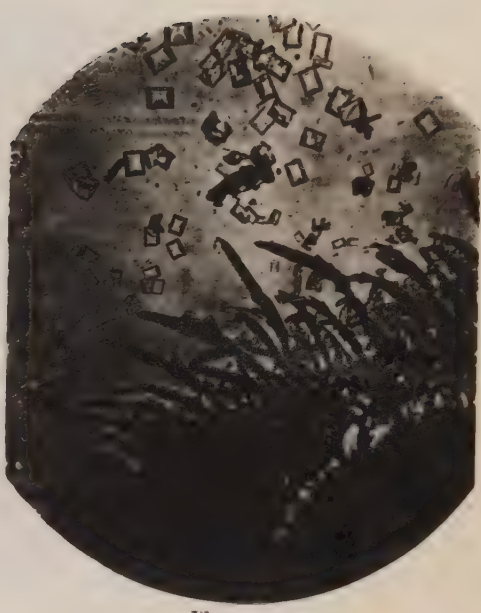

Photo. 2

Photo. 1 and 2—Ascorbic acid from suprarenal cortex, recrystd. from acetone-dioxane.

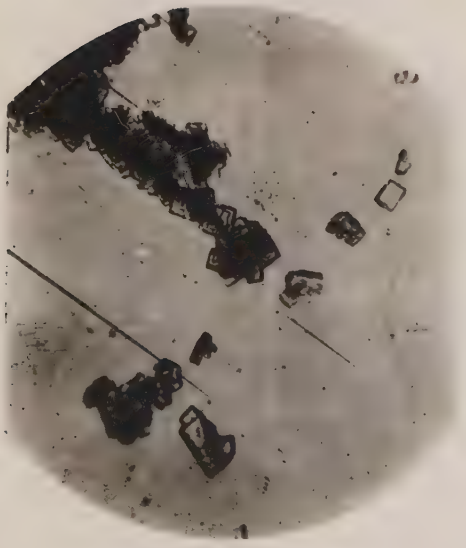

Photo. 3

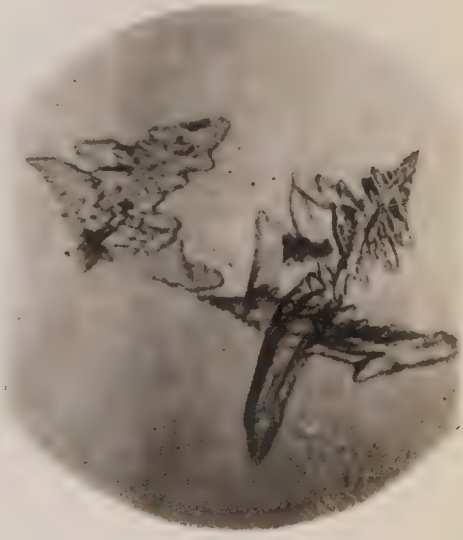

Photo. 4

Photo. 3 and 4—Ascorbic acid from Daikon juice, recrystd. from acetone-dioxane and from acetone-ether-petroleum-ether respectively.

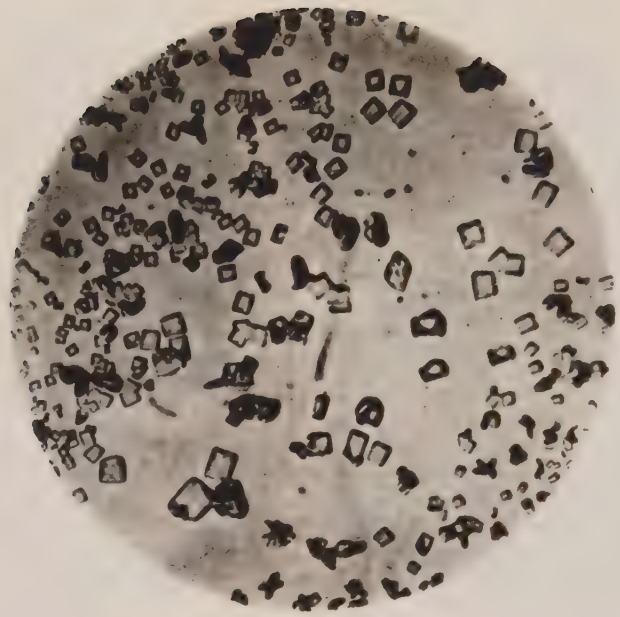

Photo. 5—Ascorbic acid from Natsumikan juice, crystals from the 1st crystallization from acetone–ether–petroleum–ether.

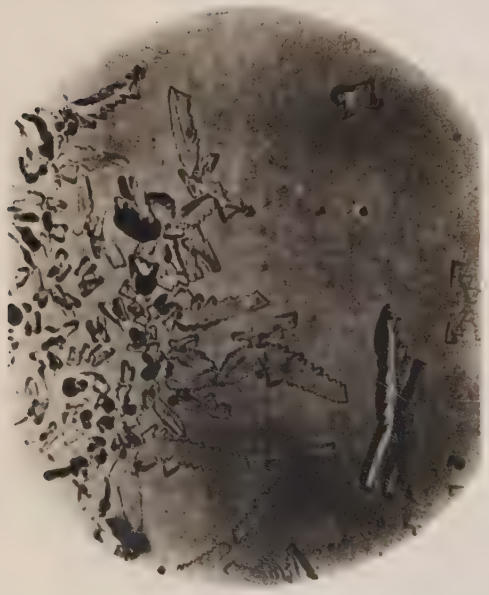

Photo. 6

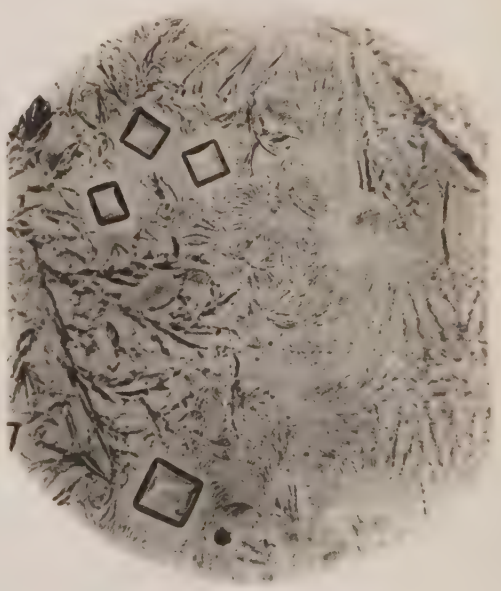

Photo. 7

Photo. 6 and 7—Ascorbic acid from Japanese green tea, recrystd. from acetone–dioxane.

purest ascorbic acid, and, therefore, it may be possible that the preparations throughly purified might be effective even in smaller quantity of dose.

Summary.

Various statements made by chemists relating to vitamin C have briefly been mentioned in order to show how the author developed its research commenced early in Feb. 1934.

Ascorbic acid has been isolated in crystalline form not only from suprarenal cortex of oxen, but also from Daikon juice (Daikon, *Rhaphanus Sativus*. L.), Natumikan juice (a kind of Japanese lemon, *Citrus Aurantium*, L. *var. sinensis*, Engl.) and Japanese green tea. By its elementary analytical values, specific rotation and other chemical and physical properties, this ascorbic acid has been proved to be identical with the existing acid.

Two therapeutic experiments were carried out with positive results parallel to each other, to examine the first two ascorbic acid preparations, namely, preparation obtained from suprarenal cortex of oxen and that from Daikon juice, for antiscorbutic activity on scurvy in guinea pigs, and the results show, so far as these preparations are concerned, that this ascorbic acid was effective in a daily dose of 0.8 mg.

Another therapeutic experiment using an ascorbic acid preparation obtained from Japanese green tea, is now in progress. So far this preparation seems to be effective also.

These evidences seem to support Szent-Györgyi's theory that vitamin C and ascorbic acid are identical.

A slight but favourable modification has been made on the method of Szent-Györgyi for obtaining ascorbic acid from suprarenal cortex.

A full description of procedure for isolating ascorbic acid is given in the case of suprarenal cortex and of Daikon juice.

In connection with the case of Natumikan juice or Japanese green tea, where isolation procedure was substantially the same as that in the other cases, are given some notes necessary for isolating ascorbic acid.

Dichlorphenol-indophenol—a reagent proposed by Tillmans as one capable of determining vitamin C in foods—has been applied in the case of suprarenal cortex and of the other vegetables in question, and, in fact, turned out to play such a rôle.

This fact confirms Tillmans' view that the vitamin C and reducing factor in vegetables are always parallel.

This work has been carried out under the supervision of Professer Umetaro Suzuki, to whom the author greatly owes. The author feels also grateful toward D. Saburo Hunahasi who accomplished all the elementary analysis.

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Kationenumtausch an den Tonen. (I. Mitteilung)

Von

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(Eingegagen am 12, September 1934)

Zur Aufklärung der Beziehung von Kationenumtausch und Struktur bei den Tonen, hat der Verfasser vier Sorten der Tonen, d. h., Bentonit, Hettenleidelheimer Fettton, Saarauer Blauton und Zettlitzer Kaolin, deren Eigenschaften von einander sehr verschieden sind, als die Versuchsmaterialien gebraucht. Sie wurden zu erst als Calciumton geändert. Dann wurden die ausgetauschte Ca-Menge gegen verschiedene Aminen mit verschiedenen Ionengrössen bestimmt. Die Ionengrösse der einzutauschender Kationen haben auf die ausgetauschte Menge des Calciums keine Einflüsse ausgeübt. Daraus hat der Verfasser so zusammengefasst, dass der Kationenumtausch an den Tonen nur auf der Oberfläche stattfinden soll.

Die Werte, die nach der Freundlichschen Formel berechnet wurden, haben mit den experimentellen werten sehr gut übereinstimmt.

Biochemical Studies on "Sotetsu", the Japanese Sago Plant. II.

On the Chemical Constituents, especially the Sex Differences of "Sotetsu"-stems.

By

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(*Kagoshima Agricultural College, Kagoshima, Japan.*)

(Received September 26, 1934.)

The places of production of the "sotetsu"-stems experimented were at Ōshima and Sata in Kagoshina prefecture; and the kinds of the samples obtained from these districts, respectively were as follows:

(1) Female stem A, which was born in 1933, and not born in 1932.
(2) Female stem B, which was born in 1932, and not born in 1933.
(3) Male stem.

These "sotetsu"-stems were studied on the both sides of layer- and sex-differences, in January, 1934.

The "sotetsu"-stem is consist of four layers that is (1) pith, (2) xylem or wood part, (3) cortex and (4) scaly part, and the ratio by weight of these parts is 10 : 20 : 30 : 40 roughly.

The "sotetsu"-stem, especially the pith and the cortex contains large amount of starch; therefore sometimes used for starch manufacture.

Experimental Results.

I. Chemical Composition of Pith and Cortex.

The chemical composition of the "sotetsu"-stem from Ōshima, was estimated as following table:

	Female stems				Male stem	
	A (born in 1933)		B (born in 1932)			
	Pith	Cortex	Pith	Cortex	Pith	Cortex
Water	81.92	78.26	75.65	78.85	83.96	78.09
Crude protein	2.11	1.70	1.51	1.69	0.81	1.52
Protein	1.09	1.48	0.88	1.30	0.67	0.81
Crude fat	0.08	0.12	0.07	0.11	0.05	0.10
Crude fibre	1.41	2.98	1.52	2.43	1.08	2.31
Crude ash	0.62	0.76	1.02	0.94	0.50	0.71

N-free extract	13.88	16.18	20.23	15.98	13.60	17.27
Total carbohydrate	12.76	13.55	18.40	13.23	12.17	14.93
Starch	5.37	5.19	9.30	6.40	6.02	8.78
Dextrin	0.78	0.87	2.74	1.60	0.56	0.65
Reducing sugar (as glucose)	2.53	3.94	1.63	2.23	1.54	2.27
Non-reducing sugar (as sucrose)	3.22	2.73	3.22	2.00	3.16	2.07

II. Sex Differences of Stem-composition.

The pith and cortex of "sotetsu"-stems obtained from Ōshima and Sata, was analysed respectively in both the male and female stems. The results were as follows:

	Samples, obtained from Ōshima						Samples, obtained from Sata		
	Pith			Cortex			Pith		
	Male	Female A born in 1932	Female B born in 1933	Male	Female A born in 1932	Female B born in 1933	Male	Female A born in 1932	Female B born in 1933
Water (%)	84.96	75.65	81.92	78.09	78.85	78.26	70.40	69.52	78.74
Dry matter	16.04	24.35	18.08	21.91	21.15	21.74	29.60	30.48	21.26
	In 100 parts of dry matter								
Crude protein	5.02	6.18	11.65	6.96	8.00	7.84	5.48	8.88	7.40
Protein	4.14	3.63	6.04	3.68	6.16	6.80	4.09	5.24	6.17
Crude fat	0.31	0.27	0.43	0.47	0.52	0.54	0.33	0.17	0.55
Crude fibre	6.72	6.23	7.78	10.53	11.51	13.71	4 48	3.65	9.82
Crude ash	3.08	4.17	3.45	3.26	4.44	3.51	3.27	3.63	4.37
N-free extract	84.87	83.15	70.55	78.78	75.53	74.40	86.44	83.67	77.86
Starch	37.52	38.21	29.71	40.08	30.22	23.88	56.14	54.96	30.43
Dextrin	3.50	11.23	4.30	2.97	7.59	4.02	3.06	6.97	1.39
Reducing sugar (as glucose)	9.58	6.70	14.00	10.35	10.55	18.12	5.25	4.08	10.22
Non-reducing sugar (as sucrose)	19.67	13.23	17.83	9.44	9.43	12.56	10.02	7.17	17.63

III. Peroxidase action of "Sotetsu"-stem.

The peroxidase action ef "sotetsu"-stems was compared with layer differences and sex differences respectively, by means of the colourimetric guaiacum tincture method.

(a) Comparison of peroxidase action of pith and cortex.

	Male stem		Female stem, A (born in 1933)		Female stem, B	
	Pith	Cortex	Pith	Cortex	Pith	Cortex
Colorimeter reading.	10 mm	5.5 mm	10 mm	5.8 mm	10 mm	4.8 mm
Ratio of cortex to 100 of pith.	100	182	100	172	100	208

(b) Comparison of peroxidase action of male- and female-stems.

	Pith			Cortex		
	Male	Female A (born in 1933)	Female B (born in 1932)	Male	Female A (born in 1933)	Female B (born in 1932)
Colorimeter reading.	10.0 mm	11.2 mm	8.5 mm	10.0 mm	15.0 mm	10.0 mm
Ratio of female to 100 of male.	100	89	118	100	67	100

Summary.

From the results of the above experiments we can summarize the following differences in chemical properties between the pith and cortex of "sotetsu"-plant.

(1) The starch content of the pith is greater than that of the cortex but in the case of male pant shows a reversed tendency.

(2) The amount of reducing sugar of the pith is less than that of the cortex. On the other hand, the non-reducing sugar content of the pith is always superior to that of the cortex.

(3) The crude fat and fibre contents of the pith are always inferior to those of the cortex.

(4) A very significant difference of the peroxidase action was observed between the pith- and cortex-juice; that is, in the juice of cortex, the enzyme action is more active than that of the pith.

Concerning the sex differences on the chemical properties of "sotetsu"-plant, we can summarize as follows:

(5) Difference of the quantity of starch between the male and female (A and B) plants were determined. In those results, there is nothing to choose between the two stems of male and female B; then in the female A, considerably small amount of starch than those of the male and female B, was estimated. This phenomena shows that the starch was consumed newly, in order to bear the seed.

(6) The dextrin content of the female B is very large than those of other stems.

(7) The content of reducing sugar of the female A was considerably greater than those of other stems. The same tendency was observed in the amount of non-reducing sugar.

(8) The protein and ash contents of the female stems in both A and B are always greater than those of male stem.

(9) The peroxidase action of the juice of female stem B is almost equal to that of male stem, but the enzyme action of female stem A is poor.